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Isolation, Purification, and Characterization of a Bioactive Metabolite from Streptomyces monomycini RVE129, Isolated from Rift Valley Soil in Hawassa, Ethiopia

Sudhamani Muddada

Koneru Lakshmaiah Education Foundation, Department of Biotechnology, Vaddeswaram, Guntur, India

Abstract

Streptomyces species have been known to produce a diverse array of bioactive secondary metabolites with significant antimicrobial and anticancer properties. In this study, we focused on the bioactive compound derived from the potent strain RVE129 and conducted its purification and characterization. We also investigated its bioactivity against various pathogens and its cytotoxicity towards human cervical cancer (HeLa) cells. The strain RVE129 was previously isolated from unexplored regions of the rift valley soil in Hawassa, Ethiopia, and its identification was accomplished through phenotypic characteristics and complete sequencing of the 16S rRNA gene. The strain was found to be closely related to Streptomyces monomycini strain NRRL B-24309 (99.65%), with accession no. (ON786620). The active fraction obtained from the strain underwent bioassay-guided purification using the TLC method after extraction with ethyl acetate. Subsequently, the compound was subjected to physicochemical and structural characterization using UV–Vis, FTIR, and NMR spectroscopic methods. Comparison of the spectroscopic data with that of known natural compounds in databases revealed that the antibiotic identified as setamycin. The purified antibiotic (RVE-02) displayed a broad spectrum of bioactivity against both Gram-positive and Gram-negative bacteria, with minimum inhibitory concentration (MIC) values ranging from 1.97 to 125 µg/ml. Furthermore, the antibiotic exhibited significant antiproliferative effects and induced morphological changes in HeLa cells, with an IC50 value of 24.30 µg/ml. These results suggest that the antibiotic obtained from S. monomycini RVE129 has the potential to combat pathogenic bacteria, including drug-resistant S. aureus. Additionally, its effect on HeLa cells indicates its promising potential as a cancer chemotherapeutic agent.

Introduction:

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The rapid increase in antibiotic-resistant pathogens and the limited availability of effective anticancer drugs have created an urgent demand for research focused on discovering new and effective antimicrobial and anticancer agents with minimal side effects [1]. Microorganisms, particularly actinomycetes, have emerged as promising sources for developing bioactive metabolites that can combat infections, cancer [2], and the toxicity associated with existing cancer treatments [3]. Streptomyces species, in particular, have been extensively studied and utilized for the production of novel compounds with diverse bioactivities. In the quest for novel bioactive molecules [4], researchers are increasingly exploring actinomycetes from unexplored and unique environments to address the challenges posed by infectious diseases and cancer [5]. Despite these efforts, the discovery of new microbial natural products has been hindered by the rediscovery of known compounds [6]. To overcome this, the poorly studied and diverse habitats of the Rift Valley in Hawassa, Ethiopia, were explored, leading to the identification of a promising actinomycete strain, RVE129, belonging to the genus Streptomyces [7].

While some studies have reported on the antimicrobial activity of actinomycetes in Ethiopia, there is a lack of published evidence regarding their antitumor activity [8]. In this context, our current research aims at purifying and characterizing antibacterial compounds from strain RVE129 and evaluating their bioactivity against drug-resistant pathogens [9]. Additionally, we investigated the in vitro cytotoxic activity of the bioactive compound RVE-02 on human cervical carcinoma (HeLa) cells [10]. These findings hold promise for the development of potential candidates to combat drug-resistant pathogens and suggest the possibility of utilizing RVE-02 as a potential anticancer agent for further investigation [11].

Materials and methods:

Microorganism and Maintenance:

The actinomycete isolate Streptomyces RVE129, known for its potential to produce antimicrobial metabolites, was previously obtained from the rhizosphere soils of the Rift Valley areas of Hawassa, Ethiopia [12]. After isolation, the strain was maintained as a pure culture on starch casein slants and stored at 4°C [13]. Bacterial test strains, including Staphylococcus aureus ATCC 259233 [14], Staphylococcus epidermidis ATCC 12228, Klebsiella pneumonia ATCC 700603 [15], Pseudomonas aeruginosa ATCC 27853 [16], Salmonella typhi ATCC 13311, Salmonella typhi ATCC 14028, and Escherichia coli ATCC 25922 [17], were provided

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by the Ethiopian Health and Nutrition Research Institute[18] (EHNRI) and were kept on Mueller-Hinton's agar slants in the refrigerator for maintenance [19].

Morphological and Cultural Characterization: The macroscopic characteristics of the strain were visually observed to determine its growth pattern, substrate [20], aerial mycelia, and diffusible pigment color by culturing[21] it on starch casein agar and International Streptomyces Project (ISP) media plates following the ISP and Bergey's manual guidelines [22]. Micromorphological features, such as spore morphology and hyphae, were identified using the coverslip method[23] and Gram staining with a trinocular light microscope at 1000x magnification[24]. Spore shape and surface were determined using a scanning electron microscope according to the method of Singh et al. [25].

Physiological and Biochemical Characterization: Various physiological tests [26], such as temperature tolerance (15-45°C), pH range (4-12) [27], and NaCl concentrations (0-10%), were evaluated on ISP2 [28] agar plates to assess the cultivation conditions for strain RVE129. Sugar utilization tests were performed [29] by observing growth on ISP-9 medium supplemented with 1.0% of different carbon sources at 30°C [30]. The isolate was screened for various biochemical characteristics, including nitrate reduction [31], gelatin degradation, starch hydrolysis, casein degradation, catalase, urease, and H2S production, as well as melanin production on tyrosine agar (ISP 7) medium [32].

Molecular-Based Analysis:

Molecular analysis involved PCR amplification and sequencing of the 16S rRNA gene at the Microbial Type Culture Collection and Gene Bank in Chandigarh, India [33]. The taxonomic identification of the strain was verified through PCR reactions using universal primers, and the 16S rRNA gene sequences were analyzed using the BLAST program to match with known sequences in the NCBI database [34]. A phylogenetic tree was constructed using the neighbor-joining approach in MEGA software version 6.0 to compare the isolate with other related type strains [35].

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Production and Extraction of Bioactive Metabolites: The potential Streptomyces RVE129 isolate was subjected to shake flask fermentation for the production of bioactive metabolites. The seed culture was prepared by inoculating spore suspensions of RVE129 in ISP2 broth medium and incubating for 3 days [36]. Subsequently, the seed culture was inoculated into cultivation medium and incubated for 10 days at 30°C. After fermentation, the culture broth was processed to extract the bioactive metabolites using ethyl acetate [37]. The extracted metabolite was concentrated under reduced pressure and stored at 4°C for further testing.

Purification of Antimicrobial Agent: The active metabolites were purified through thin-layer chromatography (TLC) using a mobile solvent technique [38]. The TLC plates were subjected to bioautography to identify the antimicrobial activity of the separated spots. Growth inhibition in the region of a spot indicated the presence of an active antimicrobial compound .

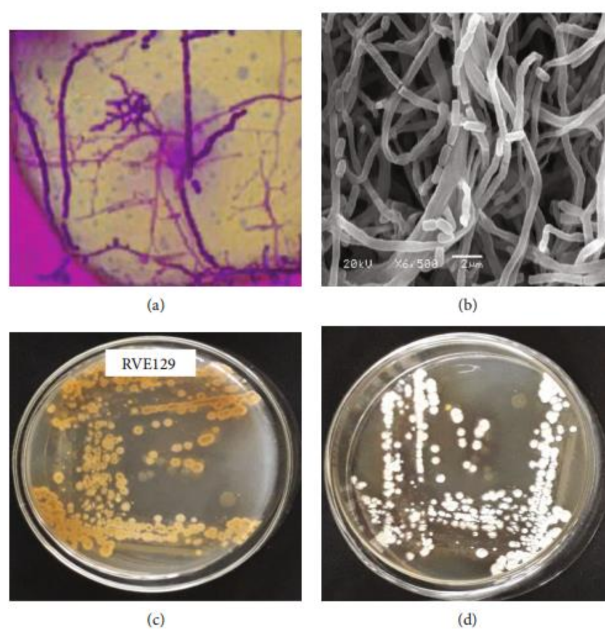

FIGURE 1: Morphological characterization of *Streptomyces* spp. RVE129 on ISP2 medium. (a) Aerial hyphae bearing spore chains under light microscope (1000x), (b) scanning electron micrograph of strains, (c) substrate mycelium, and (d) aerial mycelium.

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Table 1: The growth characteristics of the *Streptomyces* sp RVE129.

Culture medium	Growth pattern	Aerial mycelium	Substrate mycelium	Diffusible pigments
Tryptone yeast extract agar (ISP-1)	++	Grey	Pale yellow	—
Yeast extract malt extract agar (ISP-2)	+++	White	Brown	—
Oat meal agar (ISP-3)	++	White	Grey	Brown
Inorganic salt starch agar (ISP-4)	+++	Brown	Light ivory	—
Glycerol asparagines agar (ISP-5)	++	Grey	Greyish white	—
Peptone yeast extract iron agar (ISP-6)	+++	Grey	Dark grey	—
Tyrosine agar (ISP-7)	+	Cream	Brown	—
Starch casein agar	+++	White	Whitish brown	—

+++: good growth; ++: moderate growth; +: poor growth; −: no diffusible pigment.

Table 2: Morphological, biochemical, and physiological characteristics of strain RVE129.

Characteristics	Result	Characteristics	Result
Microscopic examination		Indole	—
Gram reaction	+	Voges-Proskauer	—
Spore chain morphology	Rectiflexible	*Carbon utilization*	
Motility	Nonmotile	Inositol	—
Growth	Aerobic	Mannose	+
Physiological characteristics		Xylose	±
Temperature range for growth	20-40°C	Adonitol	±
Optimum temperature	30°C	Fructose	+
pH range for growth	4 to 10	Sucrose	+
Optimum pH for growth	7	Sorbitol	+
NaCl tolerance range	0-5%	Trehalose	+
Optimum NaCl (%) for growth	2.5%	Arabinose	—
Biochemical characters		Lactose	+
Melanin production	—	Galactose	+
Nitrate reduction	—	Maltose	+
Catalase production	+	Mannitol	+
Urease production	+	Glucose	+
Esculin degradation	+	Cellulose	±
H_2S production	+	Starch	+
Citrate utilization	+	Fructose	+
Casein hydrolysis	+	Rhamnose	—
Oxidase production	—		
Gelatin hydrolysis	+		

+: positive; ±: doubtful/poor; −: negative.

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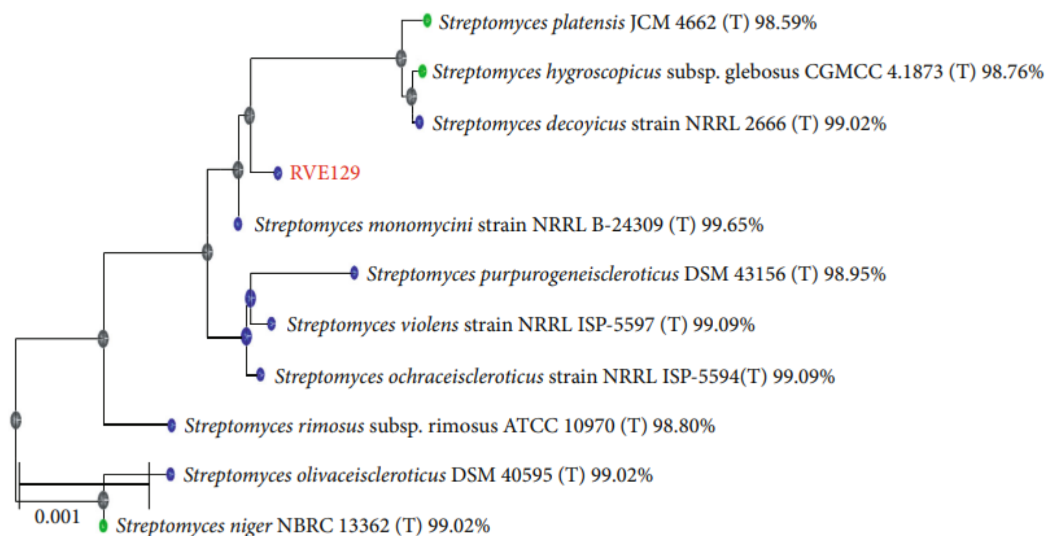

FIGURE 2: Phylogenetic tree constructed based on 16S rRNA gene sequences of RVE129. The percentage of similarity between the strain and related members of the genus *Streptomyces* 16S rRNA gene clade is shown next to the strain names.

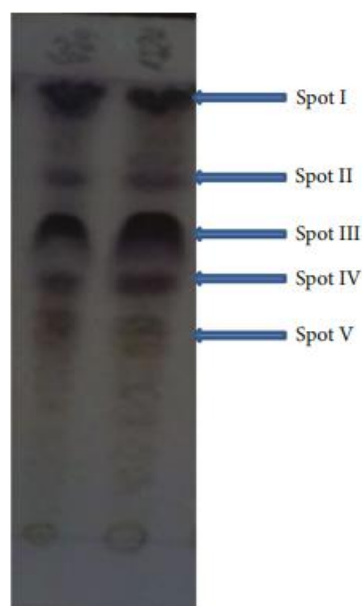

FIGURE 3: TLC chromatograph of the extract from *Streptomyces* sp. RVE129.

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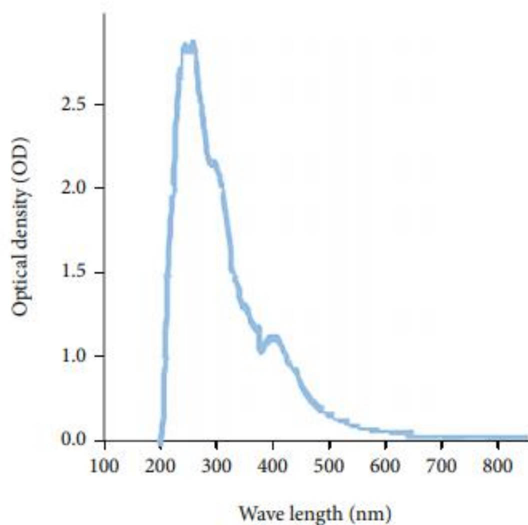

FIGURE 4: UV spectra of the purified antibiotic RVE-02.

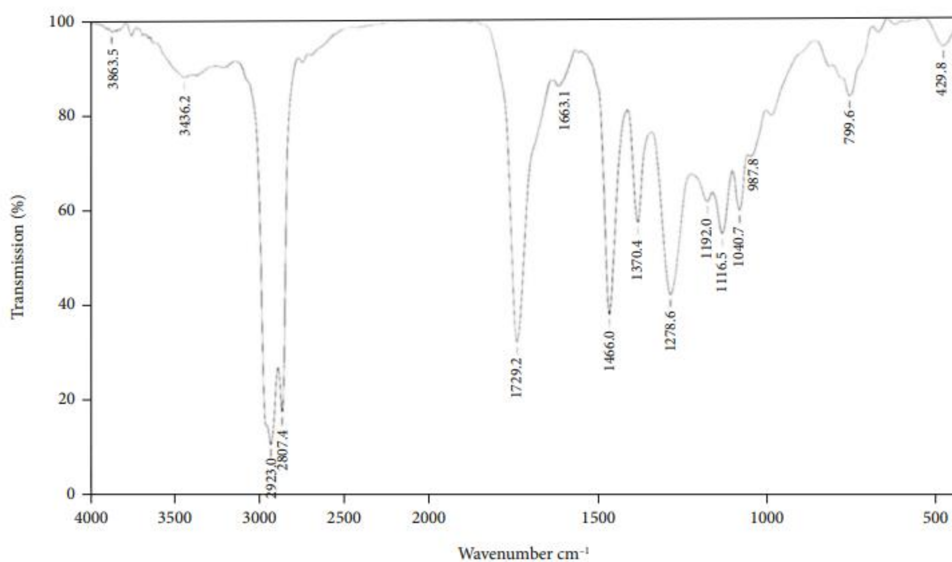

FIGURE 5: FTIR spectra of purified antibiotic RVE-02.

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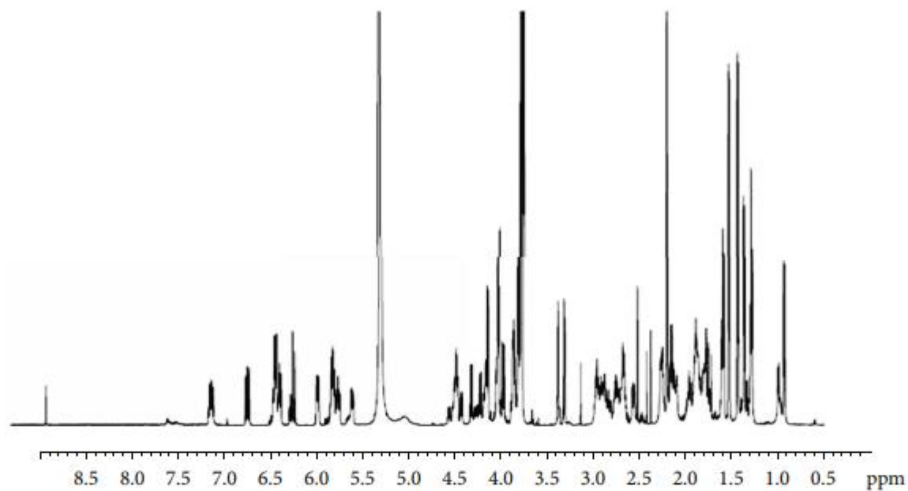

FIGURE 6: ^{1}H NMR (500 MHz, DMSO/$CDCl_3$) spectrum data of antibiotic RVE-02.

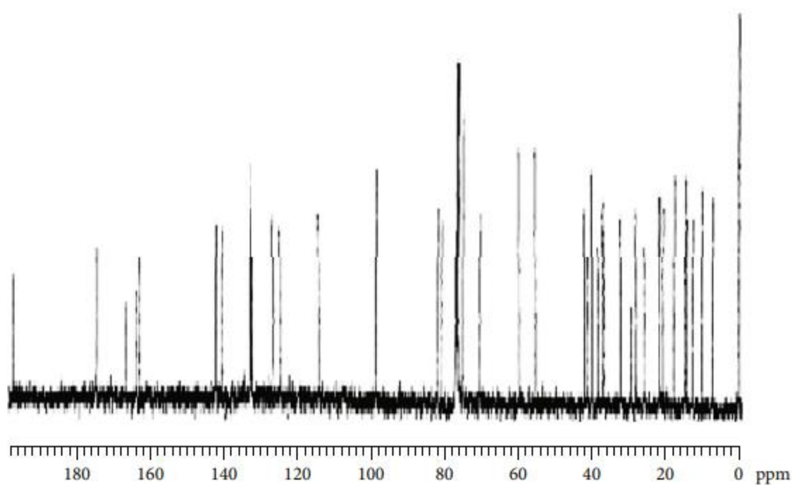

FIGURE 7: ^{13}C NMR (500 MHz, DMSO/$CDCl_3$) spectrum data of antibiotic RVE-02.

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Table 3: NMR spectra data of antibiotic compound RVE-02.

Position	δC of purified compound	δC setamycin [29]	Position	δC of purified compound	δC setamycin [29]
1	167.48	167.3	21	76.28	76.8
2	141.17	141. 2	22	40.0	40.0
2-OCH	60.02	59. 9	23	75.51	75.5
3	133.41	133.4	24	28	27.9
4	133.39	133.3	25	12.35	12.3
5	143.02	143.0	26	7.23	7.1
6	36.64	36.7	27	13.9	14.0
7	81.12	81.2	28	17.42	17.3
8	37.17	37.1	29	10.00	9.8
9	41.22	41.2	30	20.2	20.2
10	142.80	142.8	31	21.58	21.6
11	133.22	133.0	32	14.32	14.3
12	125.35	125.2	33	20.92	21.0
13	127.22	127.0	C-1′	164.29	164.3
14	70.16	70.24	C-2′	133.59	133.6
14-OCH	55.54	55.5	C-3′	133.04	133.0
15	77.36	77.2	C-4′	163.6	163.6
16	38.20	38.2	C-5′	115.02	114.9
17	82.18	82.2	C-6′	175.24	175.2
18	42.05	42.4	C-7′	26.00	25.8
19	98.42	98.2	C-8′	32.17	32.2
19-OH	—	—	C-9′	198.00	197.7
20	40.08	40.0	9′ OH	—	—

Table 4: Bioactivity potential of antibiotic RVE-02 against different pathogenic bacteria.

Test organisms	MIC (μg/ml)	Erythromycin (μg/ml)
Staphylococcus aureus ATCC-259233	1.97	1.97
Staphylococcus epidermidis ATCC-12228	3.95	1.97
Salmonella typhi ATCC-13311	31.25	7.90
Pseudomonas aeruginosa ATCC-27853	125	31.25
Kelbsiella pneumonia ATCC-700603	125	62.5
Escherichia coli ATCC-25922	62.5	7.90
Salmonella typhi ATCC-14028	62.5	7.90

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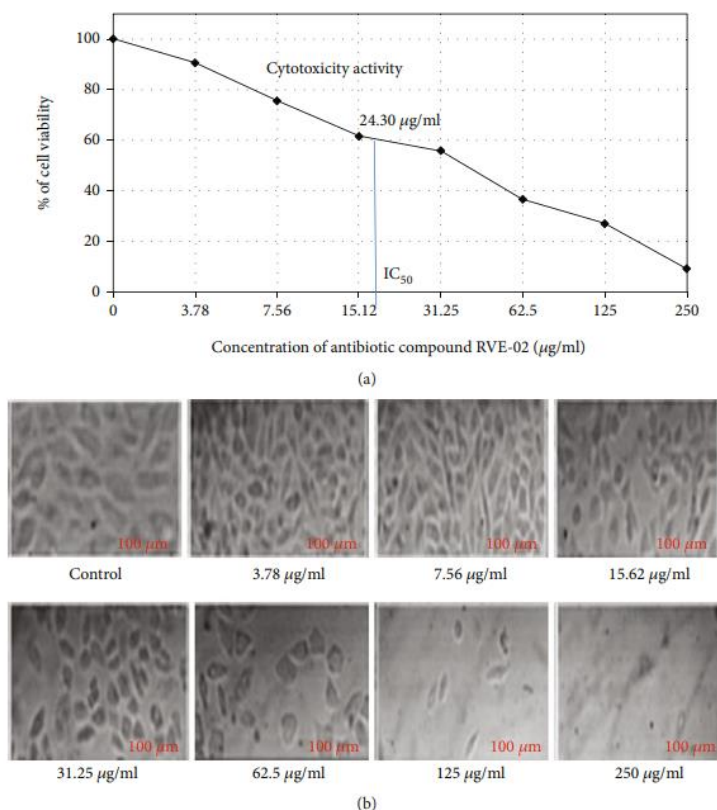

Figure 8: (a) Cytoxicity different concentrations of active metabolite on the percentage of HeLa cell viability. The data are represented as the mean ± SD of three separate experiments, for each experiment. (b) Inverted microscopic (40x) images of HeLa cell lines treated with different concentrations of antibiotic RVE-02.

Conclusion:

This study establishes that the Streptomyces isolate RVE129 exhibits high similarity to S. monomycini based on both phenotypic and genotypic characteristics. The antibiotic compound RVE-02 produced by this strain shares a close similarity with setamycin. Notably, this is the first report of the production of a polyketide antibiotic with potent antimicrobial and antitumor activity by the S. monomycini strain. The antibacterial bioactivity test demonstrated that the identified antibiotic RVE-02 is effective against a wide range of pathogens, including drug-resistant bacteria like S. aureus. Furthermore, RVE-02 displayed antitumor activity against HeLa cells in vitro, making it a promising candidate for anticancer therapy.

Data Availability

Additional data related to this study can be made available upon request from the corresponding author.

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Disclosure

The authors further certify that appropriate citations to previously reported work have been provided, and no data/tables/figures have been quoted verbally.

Conflicts of Interest

The authors declare that they have no competing interests related to this work.

Authors' Contributions

SM and DM executed, planned, and coordinated the study, confirmed the results, and reviewed the manuscript. FE designed and conducted experiments, analyzed data, and drafted the initial manuscript. BT provided technical assistance in UV, FTIR, and NMR analysis. All authors read and approved the final manuscript.

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