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Streptomyces thinghirensis and Streptomyces linomycini in Iraq: Morphological and Molecular Study

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Abstract

The current study examined the isolation and molecular characterization of *Streptomyces* spp from soil samples collected in Baghdad. Fifty soil samples were screened, yielding 41 isolates with distinct morphological and cultural characteristics. The isolates exhibited diverse aerial and substrate mycelia, pigment production, and spore chain morphologies. Genomic DNA was successfully extracted, and sequencing identified isolates as *Streptomyces thinghirensis* and *Streptomyces linomycini*. These findings confirm the prevalence of *Streptomyces* in local soils and highlight their potential as a source of bioactive compounds.

Keywords: *Streptomyces thinghirensis*, *Streptomyces linomycini*, Morphological, Molecular identification

Introduction

The name "*Streptomyces*" is derived from Greek words meaning "twisted fungus," highlighting its fungus-like, filamentous structure. The term originates from the combination of "Actinomyces" ("ray fungus") and "Streptothrix" ("twisted hair"). This genus includes aerobic, Gram-positive, non-motile, multicellular filamentous microorganisms that are commonly found in soil, where they are known as persistent saprophytes [4, 11, 6, 2].

Many *Streptomyces* species are known for their distinctive earthy scent, which is attributed to the compound geosmin. *Streptomyces* reproduce by developing threadlike hyphae that grow into surfaces to absorb nutrients. When nutrients become limited, they form aerial hyphae that fragment into spores. These spores are resilient, enabling them to survive harsh conditions and spread to new environments in search of nutrients [12].

Unlike the typical binary fission mode of reproduction, Streptomyces exhibit a complex life cycle, Initially, they develop from individual spores to a branching vegetative mycelium composed of interconnected compartments, Next, aerial hyphae, unbranched, grow in the upward direction under adverse conditions. Later, aerial hyphae give rise to chains of uni-genomic spores, which are eventually dispersed. [9].

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The International *Streptomyces* Project (ISP) provided standardized descriptions for 458 type strains of *Streptomyces*, using established species identification criteria. Subsequently, classification was further refined by incorporating physiological traits and DNA-DNA hybridization (DDH) analyses of whole chromosomal DNA as key phenotypic markers [17]. *Streptomyces* species share chemical similarities with gram-positive bacteria in their cell wall composition. Therefore, this study aimed to achieve Morphological and molecular characterization of *Streptomyces spp* bacteria.

Materials and Methods

Soil Samples Collection

A total of fifty soil samples were collected from various locations in the city of Baghdad between December 2024 and January 2025. Samples were obtained from a depth of 10 to 15 cm after removing approximately 3 cm of surface soil. The samples were placed in tightly sealed plastic bags and stored in a refrigerator. Subsequently, the soil samples were incubated at 70°C for 2 hours to eliminate non-target microorganisms. After incubation, a screening procedure was performed to isolate *Streptomyces* [7].

Isolation and Identification of *Streptomyces spp.*

Approximately one gram of dried soil was suspended in 99 ml of sterile distilled water to prepare a stock suspension. The mixture was shaken at 120 rpm for 30 minutes at room temperature. Serial dilutions ranging from 10-1 to 10-6 were prepared from the stock suspension and allowed to stand for 10 minutes. Following agitation, 0.1 ml of each dilution was pipetted onto Starch Casein Agar (SCA) supplemented with Nystatin (50 µg/L) and spread uniformly using a sterile swab. The inoculated plates were incubated at 28°C for 7 to 10 days. Colonies suspected to be actinomycetes were identified based on their small, white, pinpoint, rough, chalky appearance and the presence of a clear zone of inhibition. These colonies were further characterized by Gram staining, assessment of aerial and substrate mycelium color, and evaluation of pigment production and pigment color. Selected colonies from the initial screening (mixed culture) were transferred to separate agar plates and incubated at 28±1°C for 7 days to obtain pure cultures of *Streptomyces* species. This purification process was repeated several times. Pure cultures were stored at 4°C for further analysis [1, 10].

Molecular Identification of *Streptomyces spp*.

Extraction of Genomic DNA

A commercial extraction kit was used to extract DNA (Presto TM mini g DNA bacteria kit quick protocol, Geneaid). As per the manufacturer's instructions, DNA extraction from (G+ve) bacteria was done.

Agarose Gel Electrophoresis

The presence of PCR amplification was confirmed by agarose gel electrophoresis. The quality and integrity of the extracted DNA were deemed suitable for PCR analysis. PCR products (5 µl) were directly loaded into the wells, and electrophoresis was conducted for 1.5 hour at 70 volts/Cm. During electrophoresis, DNA fragments migrated from the negative cathode toward the positive anode. Visualization of DNA bands was achieved using a gel imaging system following staining with ethidium bromide.

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Results and Discussion

Purification and identification of *Streptomyces spp* Isolates

Isolation of *Streptomyces spp*. from dried soil samples

Fifty soil samples collected from various locations in Baghdad were screened to evaluate the effectiveness of Streptomyces as a source of antibacterial compounds. Actinomycetes and other microorganisms appeared as mixed colonies following the cultivation of diluted soil samples (10^{-1} to 10-6) for seven days on starch casein agar (SCA) medium. Fig. 1 presents small, white to grey powdery colonies suspected to be Actinomycetes isolates, including a single Actinomycete colony among mixed colonies. Suspected colonies were subcultured on SCA medium and selected based on color (grey, creamy, or white with colony diameters up to 10 mm) and morphology, which initially exhibited a smooth surface and subsequently developed a powdery, soft, and granular texture due to aerial mycelium formation. Similar findings have been reported by [6].

Out of 50 sediment soil samples, 45 (90%) were suspected to contain Streptomyces. Among these, 41 (82%) isolates displaying diverse morphological characteristics were obtained. Suspected Actinomycetes colonies were sub-cultured on SCA and ISP2 agar media to obtain pure isolates. These isolates were characterized by colored aerial and substrate mycelium, dried and rough or smooth textures, irregular or regular margins, and generally convex colonies. Most solitary colonies exhibited earthy aromas, as described [6].

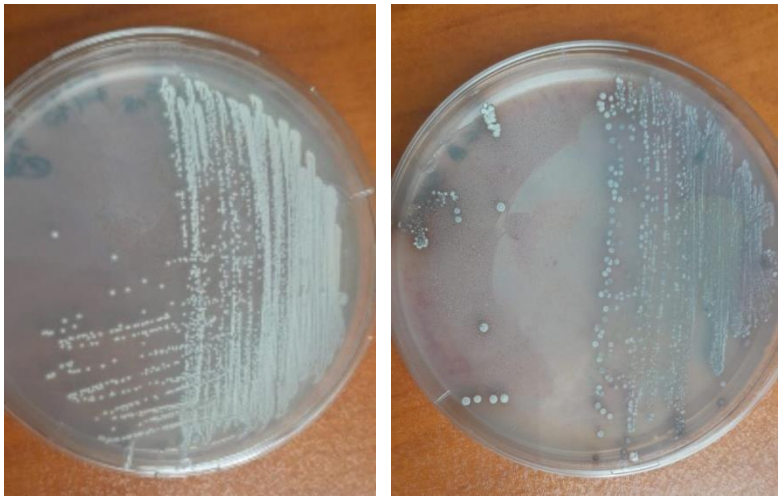

Figure 1. Initial screening of actinomycetes on casein starch agar from soil samples, incubated at 28°C for 7 to 14 days.

Selection by streaking a plate for single colonies

The streak plate method was employed to isolate and purify Actinomycetes cultures, as illustrated in Fig 2. This technique relies on successive dilution through streaking to progressively reduce bacterial concentration across the surface of an agar plate, ultimately leading to the formation of discrete colonies. A concentrated bacterial suspension is initially streaked onto a defined section of the agar surface, resulting in a high microbial load. A sterile inoculation loop or another sterilized instrument is then used to transfer a small portion of the initial streak and spread it into a new plate section. Distributing a fraction of the

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bacterial population over a larger surface area gradually reduces the bacterial density. This dilution process is repeated multiple times by streaking from one section of the plate to another, further decreasing the bacterial concentration with each pass. As a result, bacterial cells are spread thinly enough in later streaks to facilitate the development of well-separated, distinct colonies. The primary goal of this method is to obtain a single, isolated colony that can be used for further study or experimental applications. As described by (Li et al., 2016), the process is essential for obtaining pure cultures, ensuring accurate identification, and studying the morphological and biochemical characteristics of *Streptomyces* strains.

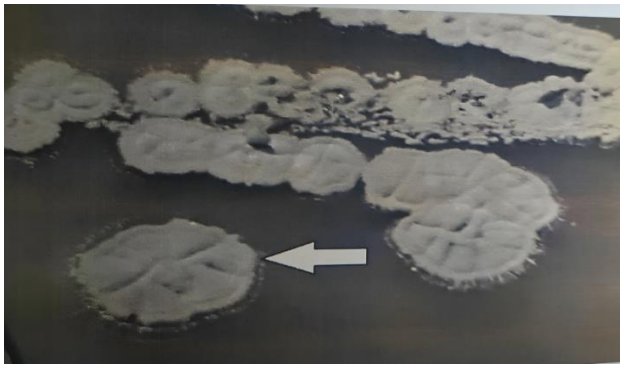

Figure 2. Formation of single colonies by *Streptomyces* spp. on Starch Casin Agar medium incubated at 28°C for 7 to 10 days using the streak plate method.

Identification and Characterization of *Streptomyces spp*.

Morphological characterization

Streptomyces isolates were identified by differences in colony shape and microscopic features, including the look of aerial and substrate mycelium, the presence of soluble pigment, and how spore chains were arranged. Some *Streptomyces spp*. isolates produced a pigmented substance that spread into the surrounding media, matching the color of the aerial mycelium. A soluble pigment was found in 20 isolates. After seven days of incubation, the isolates were examined under a microscope to observe the hyphae (see Fig. 3). The spore chain shapes varied by Streptomyces type, showing straight, spiral, or flexuous forms. Most strains exhibited a linear chain arrangement, except one, which showed a rectiflexible pattern. [8] reported identical findings in Table 1.

Table 1. Morphological features observed in *Streptomyces* isolates

Isolate No.	Colony morphology	Arial mycelium	Substrate Mycelium Reverse side pigments	Mycelium surface	Soluble pigment	Spore chain morphology
1	Irregular edge-circular	Light grey	Light brown	Smooth	brown	Straight
2	Regular edge circular	Light grey	Light brown	Smooth	Light brown	Straight
3	Irregular edge-circular	Light grey	Light brown	Smooth	Light brown	Straight

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4	Regular edge circular	Light grey	Yellowish	Smooth	Yellow	Straight
5	Regular edge circular	Grey	Darck brown	Rough	No pigment	Straight
6	Irregular edge-circular	Light grey	Darck brown	Smooth	No pigment	Straight
7	Regular edge circular	Grey	Brown	Rough	Light brown	Straight
8	Irregular edge-circular	White grey	Light brown	Smooth	No pigment	Straight
9	Irregular edge-circular	Grey	Darck brown	Rough	No pigment	Straight
10	Regular edge circular	Light grey	Light brown	Rough	light yellow	Straight
11	Regular edge circular	Light grey	Light brown	Smooth	Dark yellow	Straight
12	Regular edge circular	Light grey	Light brown	Smooth	Dark brown	Straight
13	Regular circular	Grey	Brown	Smooth	No pigment	Rectiflexible
14	Irregular edge-circular	White grey	darck brown	Rough	No pigment	Straight
15	Irregular edge-circular	pink grey	Pink	Rough	No pigment	Straight

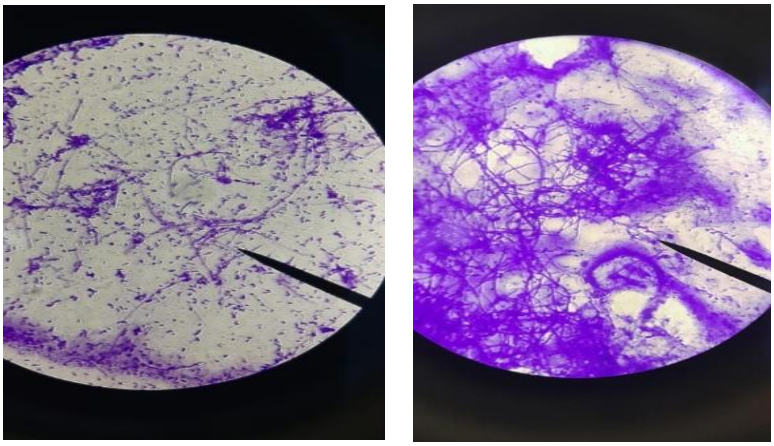

Figure 3. *Streptomyces spp*. hyphae grown on yeast extract malt extract agar (left) and yeast extract malt extract broth (right). The image shows branching filaments, abundant aerial mycelia, and long chains of small spores, which are characteristic features of *Streptomyces* spp. Magnification: 100X.

Several *Streptomyces* isolates produced diffusible pigments in the surrounding media that matched the color of their aerial mycelium. In addition, soluble pigments were detected in 15 isolates. Fig. 4 demonstrates a distinctive yellowish color, as described in Bergey's Manual of Determinative Bacteriology. [6].

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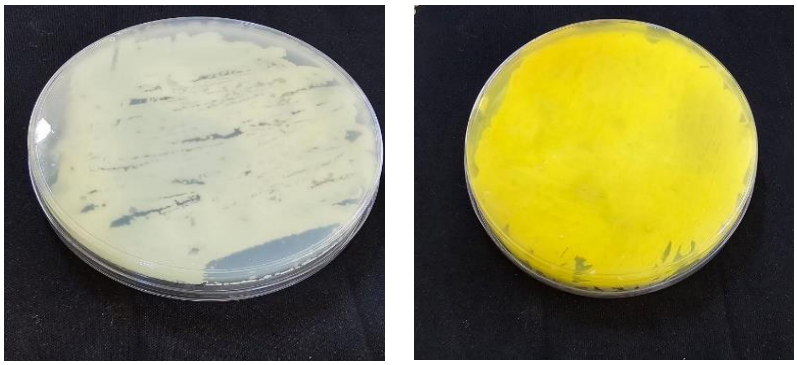

Figure 4. *Streptomyces spp*. colonies grown on Starch Casin Agar medium at 28°C for 7 to 10 days. The left isolate on the left does not produce pigment, while the one on the right produces a yellow pigment.

The mycelium surface appears rough in some species and smooth in others. The aerial mycelium exhibits coloration that may be white, dark, pale grey, or pink. The substrate mycelium is either dark brown or light brown (Fig. 5).

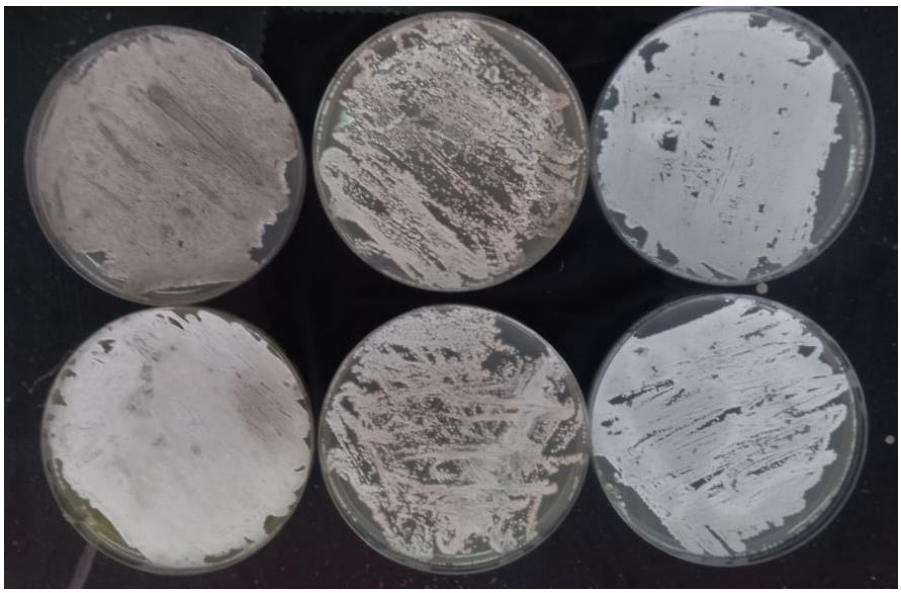

Figure 5. *Streptomyces* aerial mycelium grown on Starch Casin Agar media at 28°C for 7 to 10 days.

Molecular Assay

Genomic DNA Extraction

Whole-genome DNA was extracted from overnight cultures of two Streptomyces isolates (Af1 and Af6) using the ABIOpure TM Total DNA extraction kit. The concentration and purity of the extracted DNA were measured with a Nanodrop. Fig. 6 shows a single band of extracted DNA, which demonstrates that the extraction method was effective.

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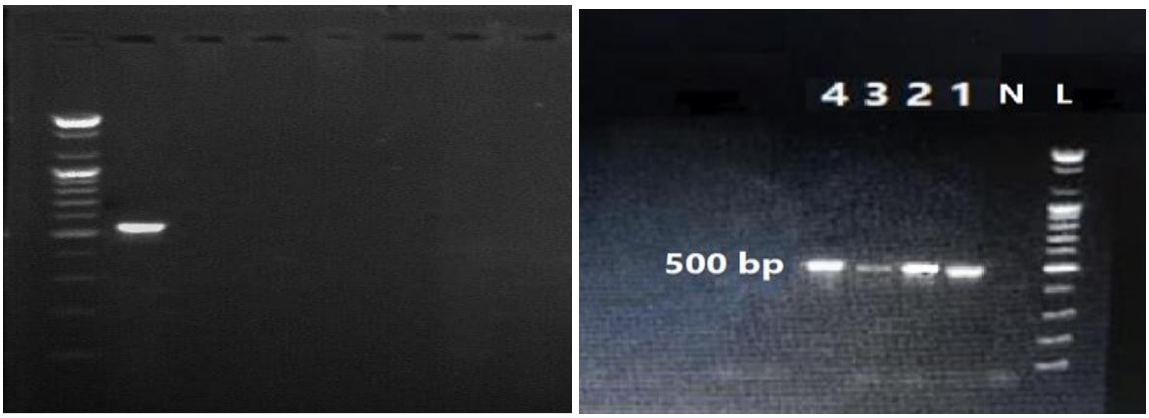

(a) (b)

Figure 6. Genomic DNA bands extracted from (a) *Streptomyces thinghirensis* (b) *Streptomyces linomycini* on (1%) agarose, 75V, for 30 min stained with ethidium bromide.

Sequencing and Alignment

Sequencing was conducted to detect *Streptomyces* spp., and PCR products were submitted to Bioneer, Korea, for sequencing. The resulting sequences (as shown in Fig.7 and Fig.8) were compared for similarity with those present in genomic databases using the NCBI BLAST program available at ncbi.nlm.nih.gov, and the most similar sequences were downloaded.

Streptomyces thinghirensis strain S10 16S ribosomal RNA, partial sequence

Sequence ID: NR_116901.1 Length: 1462 Number of Matches: 1

See 1 more title(s) See all Identical Proteins(IPG)

Range 1: 10 to 481 GenBank Graphics ▼ Next Match ▲ Previous Match

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Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
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Streptomyces lienomycini strain C.P.57 16S ribosomal RNA gene, partial sequence	Streptomyces lienomycini	787	787	96%	0.0	96.82%	1484	KF991637.1
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Streptomyces lienomycini strain P.B.21 16S ribosomal RNA gene, partial sequence	Streptomyces lienomycini	787	787	96%	0.0	96.82%	1491	KF991621.1
Streptomyces lienomycini strain F.G.B.66 16S ribosomal RNA gene, partial sequence	Streptomyces lienomycini	787	787	96%	0.0	96.82%	1491	KF991630.1
Streptomyces purpurascens strain AC1 16S ribosomal RNA gene, partial sequence	Streptomyces purpurascens	787	787	96%	0.0	96.82%	841	OP937195.1
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Streptomyces lienomycini strain C.S.66b 16S ribosomal RNA gene, partial sequence	Streptomyces lienomycini	787	787	96%	0.0	96.82%	1490	KF991639.1
Streptomyces thinghirensis strain WAE1 16S ribosomal RNA gene, partial sequence	Streptomyces thinghirensis	787	787	96%	0.0	96.82%	807	ON584359.1
Streptomyces sp. strain KC28 16S ribosomal RNA gene, partial sequence	Streptomyces sp.	787	787	96%	0.0	96.82%	1488	PP703170.1
Streptomyces purpurascens QQ10 gene for 16S ribosomal RNA, partial sequence	Streptomyces purpurascens	787	787	96%	0.0	96.82%	841	LC492892.1
Streptomyces lienomycini strain C.P.31 16S ribosomal RNA gene, partial sequence	Streptomyces lienomycini	787	787	96%	0.0	96.82%	1487	KF991628.1
Streptomyces lienomycini strain F.G.B.31 16S ribosomal RNA gene, partial sequence	Streptomyces lienomycini	787	787	96%	0.0	96.82%	1373	KF991625.1
Streptomyces sp. Q5 partial 16S rRNA gene, strain Q5	Streptomyces sp. Q5	787	787	96%	0.0	96.82%	1498	HE584615.1
Streptomyces lienomycini strain F.G.S.392 16S ribosomal RNA gene, partial sequence	Streptomyces lienomycini	787	787	96%	0.0	96.82%	1475	KF991646.1
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Streptomyces thinghirensis strain MSA1 16S ribosomal RNA gene, partial sequence	Streptomyces thinghirensis	782	782	96%	0.0	96.61%	1457	OQ457786.1

Figure 7. Sequencing of *Streptomyces thinghirensis* in NCBI

Download GenBank Graphics

Streptomyces lienomycini strain C.P.57 16S ribosomal RNA gene, partial sequence

Sequence ID: KF991637.1 Length: 1484 Number of Matches: 1

See 1 more title(s) See all Identical Proteins(IPG)

Range 1: 24 to 495 GenBank Graphics ▼ Next Match ▲ Previous Match

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Streptomyces lienomycini strain P B 21 16S ribosomal RNA gene, partial sequence	Streptomyces lienomycini	787	787	96%	0.0	96.82%	1491	KF991621.1
Streptomyces lienomycini strain C P 31 16S ribosomal RNA gene, partial sequence	Streptomyces lienomycini	787	787	96%	0.0	96.82%	1487	KF991628.1
Streptomyces rubrogriseus strain FG B 22 16S ribosomal RNA gene, partial sequence	Streptomyces rubrogriseus	782	782	96%	0.0	96.61%	1493	KF991623.1
Streptomyces thinghirensis strain MSA1 16S ribosomal RNA gene, partial sequence	Streptomyces thinghirensis	782	782	96%	0.0	96.61%	1457	OQ457786.1
Streptomyces purpurascens strain NBC_00017 chromosome, complete genome	Streptomyces purpurascens	776	4660	96%	0.0	96.59%	9188115	CP108341.1

Figure 8. Sequencing of *Streptomyces linomycini* in NCBI

Conclusion

This study demonstrated that Baghdad soils harbor diverse *Streptomyces* isolates with distinct morphological and genetic features. The successful identification of *S. thinghirensis* and *S. linomycini* underscores their ecological prevalence and potential for antimicrobial applications. Further studies on these isolates may contribute to the discovery of novel bioactive metabolites.

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Authors' Declaration

Conflicts of Interest: None.

- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images,

Authors' Contribution Statement

Afnan A. Al-saeed: Contributed to the conception and design of the study, conducted the experiments, data rearrangement and drafted the initial manuscript.

Mohsen Hashim Risan: contributed rearrangement the results, collection a part of literature review and conducted some characteristics of the products.

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