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AN EYE ON THE MOLECULAR ASPECTS OF THE ANTIBACTERIAL ACTION PRODUCED BY GLYCYRRHIZIC ACID AGAINST *E. COLI* ISOLATED FROM READY-TO-EAT MEAT PRODUCTS IN SAUDI ARABIA

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Abstract

E. coli is one of the key bacterial groups of Human microbiomes that present in the gastrointestinal tract; most isolates of it are non-pathogenic except if the host immune system is compromised then it may be considered as an opportunistic in those circumstances. Strains of *E. coli* can be involved in the pathogenesis operation of causing several diseases, such as colibacillosis in poultry and hemolytic uremic syndrome in humans. We aimed at evaluating and assessing the anti-bacterial action that resulted from using glycyrrhizic acid against *E. coli* bacteria which have been isolated from ready-to-eat meat products. The bacteria have been identified through utilization of both biochemical trials like oxidase, catalase, urease, citrate utilization and methyl red test, and polymerase chain reaction (PCR) that confirmed the identification through clarification of the band position on agarose gel. PCR was utilized for recognition of several resistance genes, for instance, ampC, TetA (A) and mphA in the isolates. The anti-bacterial ability of glycyrrhizic acid was then being evaluated at first through in vitro studies using agar well diffusion method. Obtaining of both MIC value and mean inhibition zone diameter has been done; values were 2500 µg/ml

and 13 mm, respectively. Lastly, in silico studies have been made by using molecular docking to determine the binding mode and affinity of glycyrrhizic acid with gene expression products of both blaNDM gene and OptrA gene in *E.coli*.

Keywords: *E. Coli*, Molecular Docking, Glycyrrhizic Acid, Licorice.

1. INTRODUCTION

There is global worry about the emergence and spread of bacterial struggle to widely utilized antibiotics. To safeguard both human and animal health in this area, several nations have put in place protocols for tracking resistance [1-3]. Antibiotic endurance is mostly assumed to be caused by the overuse of antibiotics [4, 5]. Gene transfer or mutations gave rise to bacterial resistance [6, 7]. Animals, people, and poultry all often have E. coli in their gastrointestinal tracts. It is a representative of the Enterobacteriaceae family and a primary source of foodborne illnesses [8]. Unhygienic slaughter procedures are the source of E. Coli contamination in meat [9*]. E. Coli* strains achieved from infected meat and meat stuffs have been revealed to be resistive to popular antibiotics [10]. Many drug-resistant genes are often present in multidrug-resistant (MDR) bacteria [11]. Human morbidity and death have increased significantly because of the speedy spread of multidrug-resilient E. Coli strains [12]. Historical texts on pharmaceutical plants provide accounts of their usage in all prehistoric cultures. Vegetable bioactive compounds have been used as substitute therapies for certain illnesses [13, 14]. The primary active ingredient in licorice root is glycyrrhizic acid. The glucouronic acid residues on glycyrrhizic acid molecule represent the hydrophilic area, whereas the glycyrrhetic acid residue represents the hydrophobic section [15, 16]. Glycyrrhizic acid is an amphiphilic molecule. Glycyrrhizic acid has been used medicinally for an extraordinarily long time. The earliest records of the use of liquorice for therapeutic purposes date back to the ancient Egyptian, Chinese, Indian, and Assyrian civilizations [16]. Numerous pharmaco-biological actions of glycyrrhizic acid include anti-inflammatory effects, suppression of coronavirus replication, and a decrease in the synthesis of pro-inflammatory cytokines by the virus [17, 18]. Following enzymatic hydrolysis, commensal microbiota absorbs glycyrrhizic acid as glycyrrhetic acid [19]. Thus, glycyrrhizic acid's pharmacological actions are the same as those of glycyrrhetinic acid [20]. GA is well recognized for its potent antibacterial properties. Its phenolic component composition is made up of flavonoids and glycosides. Additionally, it has been discovered to have immunomodulatory effects by destroying viral particles and inhibiting viral replication [21]. Numerous studies conducted in recent years have demonstrated the strong inhibitory effects of licorice aqueous, EtOH, and supercritical fluid extracts on the activities of both Gram-positive and Gram-negative bacteria, containing *Bacillus subtilis*, *E. coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Staphylococcus aureus* [22–28]. Licorice may be utilized as an alternate treatment for TB, periodontic disease, intestinal anabrosis, and dental caries, depending on their inhibitory actions against bacteria. This study aims to explore the antibacterial properties of glycyrrhizic acid against gram-negative bacteria, *i.e., E. coli*, identified by PCR, DNA sequencing, and biochemical analyses. *In vitro* studies such as the agar well diffusion test and in silico studies using molecular docking are employed.

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2. MATERIALS AND METHODS

2.1. Sampling process

A sum of 50 samples of ready-to-eat meat products were gathered from 5 several local stores (Table 1) in Taif, Saudi Arabia, from July to September 2023. The samples were placed separately in sterile bags, labeled with site of collection and sample number. Samples were transported in cooled box to laboratory for further investigation.

Table 1: Locations of local stores where samples were taken.

Location	Coordinates using GPS
1	21°16'43.9"N 40°24'42.6"E
2	21°15'45.0"N 40°25'47.0"E
3	21°17'05.7"N 40°24'58.1"E
4	21°17'10.6"N 40°26'27.1"E
5	21°16'54.0"N 40°25'05.9"E

2.2. Isolation of *E. coli* strains

Homogenization of samples was achieved using a stomacher for duration of 2 minutes. After that, 25 g from the regulated sample has been considered and placed in 225 ml of Trypticase Soya Broth added with Novobocin with a concentration of (20 mg/L) and protected for 24 h. at 37 ºC. After 24 h., 0.1 ml of the initial deepened broth was added to 10 ml of discerning upgrading EC O157: H7 broth and left at 37 ºC for 24 h. Following cultivation, a loopful of the inoculant has been sheeted on Remel MacConkey Sorbitol Agar w/ Cefixime and Tellurite and 4-Methylumbelliferyl-β-D-glucuronide hydrate agar then further incubation has been done at 37 ºC for 24 h. The pale grey green clusters with smoky centers on Remel MacConkey Sorbitol Agar w/ Cefixime agar were then exposed to different identification procedures [29].

2.3. Identification of *E. coli* strains

2.3.1. Identification using biochemical tests

Various biochemical tests have been carried out to identify the bacterial strains, tests included, gram staining technique, oxidase, catalase, citrate, indole, Voges-Proskauer test, methyl red test, urease, nitrate reduction and sugar fermentation [30].

2.3.2. Identification using polymerase chain reaction

The QIAamp DNA Mini kit (Qiagen, Germany, GmbH) was utilized to extract DNA from samples, with certain changes made in harmony with the maker's guidelines. In summary, 200 µl of the sample suspension was treated for 10 minutes at 56 OC with 10 µl of proteinase K and 200 µl of lysis buffer. 200 µl of 100% EtOH was inserted to the lysate after maturation. After that, the sample was centrifuged and cleaned in harmony with the maker's guidelines. Elution buffer containing 100 µl was utilized to elute the nucleic acid. The primers used, which came from Metabion in Germany, are mentioned in Table 2. A 25-µl reaction comprising 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer at a dose of 20 pmol, 5.5 µl of water, and 5 µl of DNA template was

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utilized to amplify the primers for PCR. A 2720 thermal cycler from Applied Biosystems was utilized to perform the process. The PCR products were separated by electrophoresis employing gradients of 5V/cm on a 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at RT. Twenty microliters of the items were put into each gel slot for the gel analysis. To ascertain the fragment sizes, a generuler 100 bp ladder (Fermentas, Germany) was used. A gel documentation system (Alpha Innotech, Biometra) took sketches of the gel, and computer software was utilized to evaluate the data.

Table 2: Primers sequences, target genes, amplicon sizes and cycling circumstances.

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			End extension	Reference
				Secondary denaturation	Annealing	Extension		
amp C	TTCTATCAAMACTGGCARCC CCYTTTTATGTACCCAYGA	550	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	[31]
TetA (A)	GGTTCACTCGAACGACGTCA CTGTCCGACAAGTTGCATGA	570	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	[32]
mph A	GTGAGGAGGAGCTTCGCGAG TGCCGCAGGACTCGGAGGTC	403	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	[33]

2.4. Evaluation of anti-bacterial effects of glycyrrhizic acid

In harmony with the guidelines provided by the National Committee for Clinical Laboratory Standards (NCCLS), the susceptibility tests were conducted. The inhibitory zone screening tests were conducted using the well diffusion technique. After colonies were developed overnight on an agar plate, the inoculum suspension was made and added to Mueller-Hinton broth. Mueller-Hinton agar plates were injected with the solution using a sterile swab that had been submerged in it. To find the minimum inhibitory concentration (MIC), glycyrrhizic acid was dispersed in dimethyl sulfoxide (DMSO) at various doses (10, 5, 2.5 mg/ml). After 24 hours at 37 oC, the inhibition zone was tested around each well [34].

2.5. *In silico* studies using molecular docking

To investigate the binding mode between different bacterial proteins, blaNDM and optrA for *E. coli* and Glycyrrhizic acid at the molecular level, the proteins with the PDB ID were selected (Table 3). The template Chemical structure of Glycyrrhizic acid (GLA) was retrieved from drugbank database (DrugBank Accession Number: DB13751). To investigate the molecular interactions between ligands and proteins, Autodock Vina 1.5.6 was utilized to make all protein structures for docking using AutoDockTools (ADT). Water molecules were then removed, and the macromolecules were transformed to Autodock

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PDQBT format, where Gasteiger charges were augmented to each atom, non-polar hydrogens were merged, atomic partial charges were re-distributed, and atom types were determined. After loading the ligands (GLA) into ADT 1.5.6, the program automatically mapped the atom types and estimated Gasteiger charges. Following the determination of the torsion number and root, the ligands were formatted in Autodock PDBQT. The grid size was set to 20 20 20 with a spacing of 1, and the exhaustiveness option was left at its default setting. The binding site was established *via* the ligand core included in the PDB structure. Furthermore, Ligplot was used to construct a 2D structure and Pymol was used to display 3D binding interactions [35].

Table 3: Selected proteins and their PDB ID.

Protein	PDB ID
blaNDM	5zr8
OptrA	Uniprot code: A0A1W5LD39_ENTFL

3. RESULTS

3.1. Isolation of *E. coli* strains

After incubation using both, Remel MacConkey Sorbitol Agar w/ Cefixime and Tellurite and 4Methylumbelliferyl-β-D-glucuronide hydrate agar, number of *E.coli* strains isolated was obtained (Table 4).

Table 4: Number of *E. coli* strains isolated.

Number of samples	Number of *E. coli* isolated
50	17

3.2. Identification of *E. coli* strains

3.2.1. Biochemical tests

A variety of biochemical examinations were performed to identify the bacterial strains and the biochemical interactions results were noted (Table 5).

Table 5: Biochemical examinations executed to identify the bacterial strains.

Biochemical tests	Results
Urease test	-ve
Methyl red test	+ve
Catalase	+ve
Citrate consumption test	-ve
Indole test	+ve
Triple sugar iron test (H_2S production)	+ve
Voges- Proskuer test (VP)	-ve
Oxidase test	-ve
Sugar fermentation test	+ve
Nitrate reduction test	+ve

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3.2.2. *Polymerase chain reaction*

The PCR products fragment sizes which are ampC, TetA(A) and mphA were observed on 1.5% agarose gel after being separated using electrophoresis (Figure 1). Determination of the fragment sizes was done using a generuler 100 bp ladder, then the gel was snapped via gel recording system.

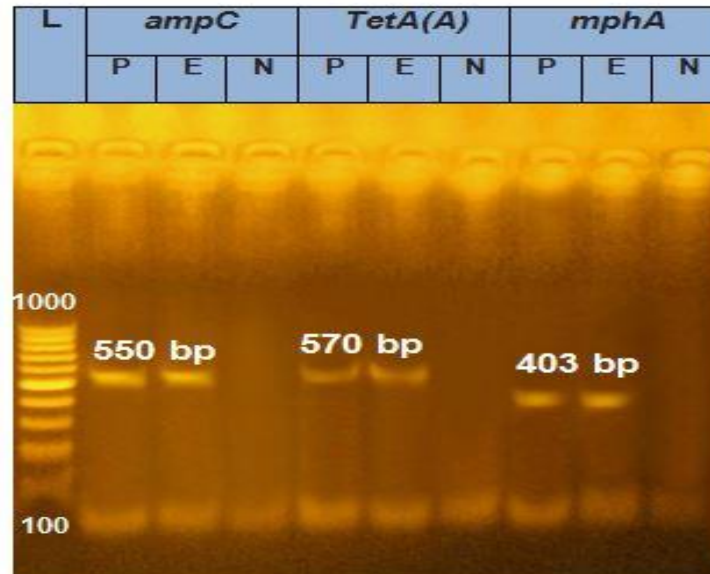

Figure 1: Agarose gel image of the fragments size of the PCR products after separation using electrophoresis.

Image displaying the band position of detected genes which include ampC, TetA(A) and mphA. The band positions were 550 bp, 570 bp and 403 bp, respectively.

3.3. Evaluation of anti-bacterial activity of glycyrrhizic acid

The goal of the research was to measure and assess the anti-bacterial activity of glycyrrhizic acid. Agar well distribution assay was executed for the sake of completion of that goal and the values of inhibiton zone diameter and MIC were recorded (Table 6) (Figure 2).

Table 6: Glycyrrhizic acid anti-bacterial effect in terms of inhibition zone and MIC values

Tested microorganism	Inhibiton zone diameter (mm)	MIC(µg/ml)
E. coli	13	2500

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Figure 2: Inhibiton zone diameter of glycyrrhizic acid against *E.coli*.

3.4. Molecular docking

Molecular docking has been executed to interpret the binding mode and affinity of glycyrrhizic acid with the proteins resulted from the gene expression process of blaNDM and OptrA genes. After completion of the molecular docking, the results were obtained. The 2D and 3D binding confirmations of glycyrrhizic acid with the active site of the protein produced through gene expression of blaNDM have been obtained (Figures 3&4).

Moreover, results of docking with OptrA are listed in figures below (Figures 5&6).

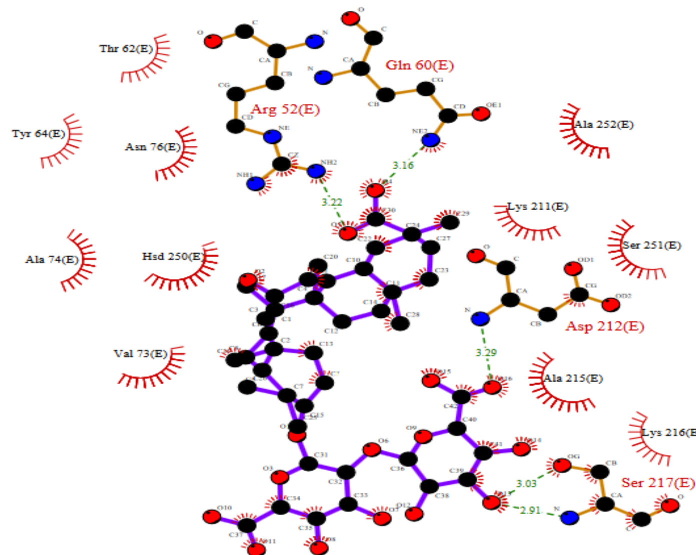

Figure 3: The 2D binding conformation of Glycyrrhetic acid in the active site of blaNDM. It makes 1 hydrogen bond with ASp(E),1 hydrogen bond with Gln 60 (A),1 hydrogen bond Arg 52(E), and 2 hydrogen bonds with ser 217 (E) with the red stand for the large hydrophobic region consisting of amino acid residues.

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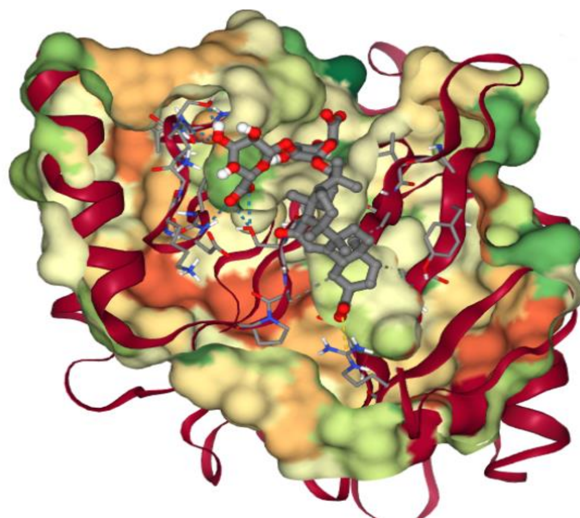

Figure 4: The 3D binding conformation of Glycyrrhetic acid in the operational location of blaNDM.

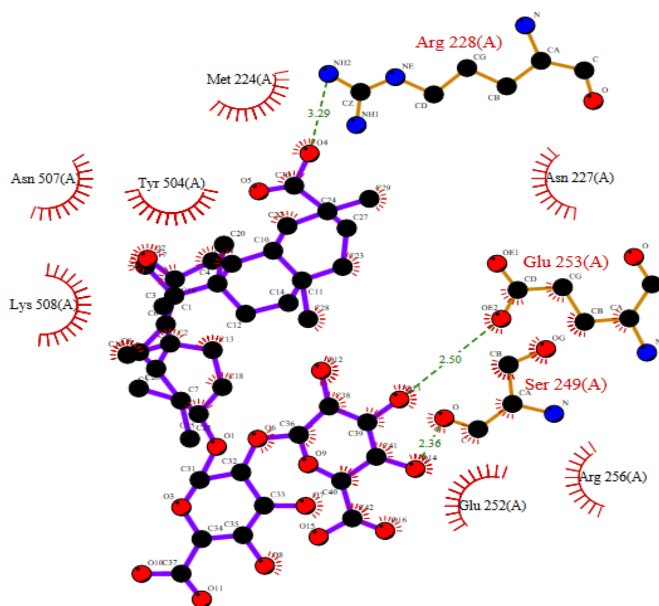

Figure 5: The 2D interaction conformation of Glycyrrhetic acid in the active site of optrA. It makes 1 hydrogen bond with Glu 253(A), 1 hydrogen bond Arg 228(A), and 1 hydrogen bond with ser 249 (A) with the red stand for the large hydrophobic region consisting of amino acid residues.

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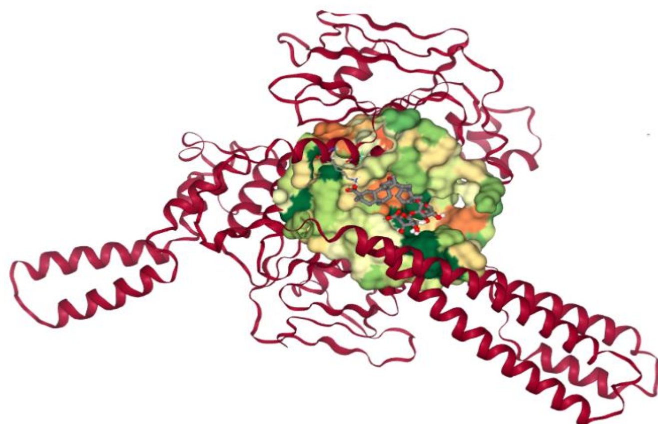

Figure 6: The 3D binding conformation of Glycyrrhetic acid in the active site of OptrA.

4. DISCUSSION

Gram-negative, facultatively anaerobic, rod-fashioned, and very motile bacterium, *E. coli* is a common resident of both animal and human intestines. It is a member of the Enterobacteriaceae family [36, 37]. *E. coli* is known for its ability to cause several diseases in immunocompromised patients when acting as an opportunistic pathogen, for instance, meningitis, endocarditis, urinary tract infection and septicemia [38]. Because of the potential existence of enteropathogenic and/or toxic strains that cause a broad range of enteric and extraintestinal disorders in animals, its recuperation from food may be concerning for public health [39, 40]. In this research, we have experimented and evaluated the anti-bacterial effect of glycyrrhizic acid on *E. coli* bacterial strains which have been extracted from ready-to-eat meat stuffs in Saudi Arabia. The isolation procedures were done through enrichment medium and were quietly like the procedures reported in another related research [41]. The identification procedures were involved execution of a number of biochemical tests such as catalase, oxidase, urease, citrate utilization and methyl red test, the biochemical identification approach execution was identical to what have been used before in another research [42]. Furthermore, PCR was performed to authorize the identification of the bacterial strains through detection of several resistance genes like ampC, TetA (A) and mphA, after observation of the resultant PCR-items on the 1.5% agarose gel, the bands position was obtained for each gene and the values were 550 bp for ampC, 570 bp for TetA and 403 bp for mphA. The utilization of PCR for the sake of identification of different microorganisms has been of vital importance as many studies have involved it to achieve correct and precise identification results [43]. Regarding assessment of the anti-bacterial activity of glycyrrhizic acid, we have completed a series of in vitro and in silico investigations. In vitro reports were involved the implementation of agar well diffusion method to obtain both of MIC and inhibition zone diameter values; values were 2500 µg/ml and 13 mm respectively, the use of agar well diffusion assay to evaluate the anti-bacterial effects of glycyrrhizic acid in this

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research is highly coincided with the steps performed to evaluate other substances in former research [44]. Furthermore, in silico studies were performed to mimic the binding mode of glycyrrhizic acid with several proteins which resulted from the gene expression process of genes like blaNDM and OptrA genes. The binding mode for glycyrrhizic acid with blaNDM showed 1 hydrogen bond with ASp(E),1 hydrogen bond with Gln 60 (A),1 hydrogen bond Arg 52(E) and 2 hydrogen bond with ser 217 (E) with The red stand for the large hydrophobic region consisting of amino acid residues while the result of docking with OptrA displayed 1 hydrogen bond with Glu 253(A), 1 hydrogen bond Arg 228(A), and 1 hydrogen bond with ser 249 (A) with The red stand for the large hydrophobic region consisting of amino acid residues. A lot of preceding studies have evaluated different activities of glycyrrhizic acid through molecular docking approach and it is highly effective in assessment of variety of uses for different substances [45-47].

5. CONCLUSION

We perform this study for the sake of assessment and evaluation of the anti-bacterial effect of glycyrrhizic acid against *E. coli* strains which were extracted from ready-to-eat meat stuffs in Saudi Arabia. Identification has been completed biochemically through running several biochemical examinations, for example, catalase, oxidase, urease, citrate utilization and methyl red test. Moreover, polymerase chain reaction (PCR) was utilized to validate the identification process through detection of several resistance genes like ampC, TetA(A) and mphA and displaying their fragments size on the agarose gel. The anti-bacterial activity that resulted from glycyrrhizic acid was being assessed by agar well diffusion method and both of MIC value and mean inhibition zone diameter were obtained. In addition to that, molecular docking approach was used to mimic the interacting mode and affinity of glycyrrhizic acid with both of blaNDM and OptrA gene expression products in *E. coli*.

Data Availability Statement

The study's original contributions are involved in the article Material; extra inquiries should be handed over to the relevant author.

Conflict of Interest Statement

According to the authors, there were no financial or commercial ties that would raise the possibility of a conflict of interest while conducting the study.

Author Contributions:

Conceptualization: Fayez Althobaiti, Eldessoky S. Dessoky; Formal analysis: Fuad A. Alatawi, Doaa Bahaa Eldin Darwish; funding acquisition: Fayez Althobaiti, Eman M. Sharaf, Eldessoky S. Dessoky; Investigation: Sanaa Almowallad, Fuad A. Alatawi, Maha Mohammed Alharbi; Methodology: Mohamed Y. Omara, Muneefah Abdullah Alenezi, Maha Mohammed Alharbi; Project administration: Eman M. Sharaf, Fayez Althobaiti, Eldessoky S. Dessoky; Resource: Fauzeya Mateq Albalwe, Wedad Ahmed, Doaa Bahaa Eldin Darwish; Supervision: Eman M. Sharaf, Doaa Bahaa Eldin Darwish; Validation: Sanaa Almowallad, Wedad Ahmed; Visualization: Fauzeya Mateq Albalwe, Muneefah Abdullah Alenezi; Writing original draft: Mohamed Y. Omara, Sanaa Almowallad; Writing – review & editing: Mohamed Y. Omara.

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