

A NOVEL APPROACH IN USING *E. ELATERIUM* NANOPARTICLES AS A NATURAL ANTIMICROBIAL AGENT COMBATING MULTIDRUG-RESISTANT *STREPTOCOCCUS PYOGENES*

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ABSTRACT

Every day, the number of deadly pathogenic multidrug-resistant (MDR) *S. pyogenes* increases so posing a serious threat to human health. This study's goal was to determine and measure *S. pyogenes* in clinical samples using serotyping. The *mefA* gene, which is responsible for Macrolide resistance, the *parC* gene, which is responsible for Quinolone resistance, and the *tetO* & *tetM* genes, which are responsible for Tetracycline resistance, were detected. Nano *E. elaterium* was used as an all-natural treatment for MDR *S. pyogenes* strains. Eighty specimens were found to have *S. pyogenes*. 22 out of 80 samples (27.5%) had multidrug resistance. On the other hand, males were probably more susceptible to infection. 36.36% of *S. pyogenes* isolates had the four genes, whereas 86.36% had the *mefA* gene, 77.27% had the *parC* gene, 77.27% had the *tetO* gene, and 86.36% had the *tetM* gene. For *E. elaterium* nanoparticles, the MIC was determined to be 20µg/ml. The cytoplasmic structures, nuclear material, and cell wall of MDR *S. pyogenes* were all harmed by *E. elaterium* nanoparticles. Additionally, they had a greater impact on the human gastric epithelial cell line's (GES1) cell viability than the gentamicin drug. Gentamicin was more cytotoxic to GES1 normal cells than *E. elaterium* nanoparticles, as evidenced by the IC50 of the drug being 190.0±8µg/ml and that of the *E. elaterium* nanoparticles being 1220.0±73µg/ml. *E. elaterium* nanoparticles are safer than gentamicin and have a strong impact against MDR *S. pyogenes*.

Keywords: *Ecballium elaterium*, Multidrug-resistant, Nanoparticles

INTRODUCTION

Gram-positive *Streptococcus pyogenes* (GAS; also called group A *Streptococcus*) is responsible for a number of infectious diseases, such as invasive infections (necrotising fasciitis, bacteremia, and meningitis), superficial infections (purulent tonsillitis) (Brouwer *et al.*, 2023). GAS secretes several virulence agents that harm host cells, tissues, and the immune system. For instance, the M protein of GAS enters epithelial or endothelial cells by binding to fibronectin (Fn) on the host cell's surface. This process is thought to be essential to GAS's evolutionary success and flexibility. In order to penetrate deeper tissue areas and cause serious infections, GAS must elude the host's innate immune system after colonisation (Wrighton *et al.*, 2023).

Streptococci strains frequently exhibit significant levels of tetracycline (TET) resistance (Gizachew *et al.*, 2019). In *streptococci*, MGEs often carry erythromycin resistance determinants and genes encoding resistance to TET (Cattoir, 2016). The high incidence of resistance may be explained by conjugative transposons' ability to easily translocate among related bacteria and their existence of tetracycline resistance determinants (*tet* genes) (Santoro *et al.*, 2014). The quinolone resistance determining region (QRDR) of topoisomerase IV ParC is the primary site of point mutations that cause fluoroquinolone (FQ) resistance. FQ resistance in *S. pyogenes* is said to be inherited from either *parC* or *gyrA* and to develop gradually. Initially, mutations in *parC* may result in low-level resistance. In addition to occurring naturally, horizontal gene transfer from other streptococcal species can also result in mutations in the *parC* QRDRs (Shen *et al.*, 2018).

Ecballium elaterium has long been utilized for a variety of therapeutic uses. The plant, often referred to as squirting cucumber, is found throughout the Mediterranean region. Greenish-yellow blooms and 30-100 cm tall stems characterize this perennial, meaty, rough-hairy shrub (Salhab, 2013). Traditional medicine has made considerable use of this plant to treat a variety of conditions, including constipation, jaundice, and rheumatism (Bohlooli *et al.*, 2012). The fruit's fresh juice has also been smeared into the nose to cure rheumatism, sinusitis, and chronic jaundice (Salhab, 2013).

According to Gómez-Núñez *et al.* (2020), nanoparticles (NPs) have many modes of action against bacteria, which increases their capacity to harm various prokaryotic structures simultaneously and hence increase their overall antibacterial efficacy.

The purpose of this investigation was to identify and measure *S. pyogenes* from clinical samples by biochemical reactions and serotyping method especially that has the multidrug-resistant (MDR), with the detection of *mefA*, *tetO*, *tetM* and *parC* genes responsible for MDR in *S. pyogenes* and the use of *E. elaterium* extract and nanoparticles as an all-natural treatment for MDR *S. pyogenes*. Additionally, *E. elaterium* nanoparticles cytotoxic impact on the GES1 normal cell line in comparison to the preferred medication was the aim of the investigation.

MATERIAL AND METHODS

Sample collection and bacterial identification

Distinct clinical specimens (sputum and blood specimens) (80) were collected during October 2022 to June 2023 From Mbarret El-Asafra Hospital in Egypt. The patients were between the ages of 18 and 45. This study examined the association between age, gender, and the prevalence of *S. pyogenes* infection in both male and female patients.

Samples of sputum were gathered in a sterile, leak-proof container, inoculated onto Oxoid, England's Blood Agar plates, and then incubated at 37°C for the whole night (Spellerberg, 2016). Blood samples were collected from patients where Blood (1-2ml) was collected into 10ml of nutrient broth (Oxoid, England) with 0.05% sodium polyanethol sulphonate. The broth was incubated at 37°C, overnight then subculture on blood agar plate (Ravichitra *et al.*, 2014).

First, the bacterial identification was confirmed using the VITEK 2 system from BioMerieux in the US (Henning *et al.*, 2015). To identify Streptococcal Groups, particularly *S. pyogenes*, a confirmation test was conducted using the serotyping technique (LK06-HiStrepTM Latex Test Kit, India) (Hall and Beiko, 2018).

Detection of multidrug-resistant *S. pyogenes*

The susceptibility of the *S. pyogenes* isolates to twelve wide spectrum antibiotics from nine distinct classes was evaluated, including ampicillin (AM 10µg), meropenem (MEM 10µg), azithromycin (AZM 15µg), cefaclor (CEC 30µg), cefoperazone (CEP 75µg), cefepime (FEP 30µg), ciprofloxacin (CIP 5µg), nalidixic acid (NA 30µg), trimethoprim/sulphamethoxazole (SXT 1.25/23.7µg), chloramphenicol (C 30µg), tetracycline (TET 30µg) and gentamycin (CN 10µg)

(Oxoid, England). Antibiotic discs were initially placed on Mueller-Hinton agar plates 0.5 McFarland inoculum that had been rapidly developed. After that, the plates were incubated for a further twenty-four hours at 37°C. Following that, the inhibitory zone's breadth was weighted and compared to the CLSI guidelines (Humphries et al., 2021).

Detection of *mefA*, *parC*, *tetO* and *tetM* genes using *S. pyogenes* DNA

A QIAamp DNA Mini Kit (Cat. Nos. 51304 and 51306, QIAGEN, USA) was used to extract the bacterial DNA fragment. *S. pyogenes* was newly cultivated for 24 hours at 37°C on a 5ml nutrient broth medium (Oates et al., 2012).

Polymerase chain reaction analysis is used to identify the genes responsible for Macrolide resistance (*mefA*), Quinelone resistance (*parC*), and Tetracycline resistance (*tetO* & *tetM*). Germany's Metabion International AG delivered the primer pairs (Table 1).

Table 1 The particular primer sequences utilized to identify the genes *mefA*, *parC*, *tetO*, and *tetM*

Primer		Sequence (5'-3')	Tm °C	Product size (bp)	Ref.
<i>mefA</i>	Forward	CAGGGTCATAAAGCCTAAATAG	60.0°C	432	Rubio-López et al., 2012
	Reverse	GAGGTAAGCTACATAAACTGTG	59.9°C		
<i>tetO</i>	Forward	ACGGARAGTTTATTGTATACC	52.6°C	171	Dundar et al., 2010
	Reverse	TGGCGTATCTATAATGTTGAC	53.5°C		
<i>tetM</i>	Forward	ACAGAAAGCTTATTATATAAC	54.2°C	171	Dundar et al., 2010
	Reverse	TGGCGTGTCTATGATGTTTAC	56.6°C		
<i>parC</i>	Forward	GGATTGAAACCCGTTTCAGCG	59.9°C	429	Rivera et al., 2005
	Reverse	CTGGTAAAACGGTGGGTTCT	60.1°C		

The PCR was replicated using a 25µl master mix (Promega, USA), forward and reverse primer (10pmol/l) 2µl for each one, and 5µl of extracted DNA in a 5µl volume treated with sterile H₂O DEPC. Utilizing the Veriti 96-well Thermal Cycler from Biosystem in the United States, the cycling conditions for gene identification were established (Su et al., 2021).

The results of the PCR were then electrophoresed on a 1.5 per agarose gel (produced by Vivantis, USA) (Bio-Rad Laboratories, Hercules, CA, USA). Two microliters of 10mg/ml Ethidium bromide were then used to stain the gels. To analyze the data, the MultiDoc-ItTM system (UVP-gel documentary system) was utilized (www.totallab.com, Ver.1.0.1). Using SYNGENE (680XHR, U.K.) and spectrophotometry, the purified PCR results were evaluated at 312 nm (Hall and Beiko, 2018).

Preparation of *E. elaterium* extract and nanoparticles

E. elaterium was discovered at the National Research Center in Egypt after being acquired from El-sheikh Zowaid in North Sinai. Samples of fresh plants were allowed to air dry for seven days at ambient temperature (28°C) in a dark place (Saker et al., 2012). An equipment for grinding ground the dry herbs into a powder (Ahmadi et al., 2017). According to Sedighi et al. (2013), 200ml of room-temperature sterile distilled water was added to 50g of dry components, and the mixture was left for 72 hours.

E. elaterium was gathered, repeatedly washed with deionized water, and dried at 28°C. A ball milling machine (TENCAN, China) was used to turn them into a powder. Five grams of *E. elaterium* were processed into very fine particles using nanotubes for four hours as part of the grinding process. Localized high pressure would result from the small, rigid balls colliding during the ball milling process in a hidden container (Rabiee et al., 2020).

Synthetic nanoparticles characterization

Characterization techniques were applied according to Rabiee et al., (2020). A particle size distribution of *E. elaterium* was measured using DLS (Dynamic Light Scattering), Nicomp Nano Z3000 System (Entegris Ltd, USA). 10mg of *E. elaterium* nanoparticles was added to 5ml distilled water, incubated for 5 min. and measured at 23°C by DLS.

A TEM (Transmission Electron Microscope) was used to examine the size and shape of *E. elaterium* nanoparticles (JEOL JEM-1400 series TEM, Japan). After dissolving 1mg of Nano *E. elaterium* in 10ml of D.W., 2µl droplets of the nanoparticles were deposited onto parafilm and transferred straight onto electron microscopy (EM) grids. After wicking away the specimen drop using the filter paper, it was put in a Petri dish.

Antibacterial screening of MDR *S. pyogenes* using *E. elaterium* extract and nanoparticles

The antibacterial capabilities of various solvent extracts and nanoparticles have been evaluated using the agar well diffusion method. The agar surface was covered with filter paper discs, each of which held 50µl of the extract solution and had a diameter of around 6 mm. Depending on the test microorganism, agar plates are then stored at 37°C the next day under the appropriate circumstances. The growth-inhibitory zones' diameter was measured (Daoud et al., 2019).

To assess their effects, *E. elaterium* nanoparticles were serially diluted and added to MDR *S. pyogenes*. *E. elaterium* nanoparticles produced in D.W. were diluted to measure the MIC and the MBC (1, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100µg/ml) (Macé et al., 2017). 5µl of microbial inoculum was added to 100µl successive *E. elaterium* nanoparticle dilutions in tubes containing 95µl of nutritional broth. The bacterial suspension in negative control tubes does not contain *E. elaterium*

nanoparticles, whereas the *E. elaterium* nanoparticle suspension is the sole substance in positive control tubes. Each tube was incubated at 37°C overnight. MIC and MBC values were determined spectrophotometrically (5010 V5+, Germany) at 600 nm (Mutlu-Ingok et al., 2021).

Detection of the impact of *E. elaterium* nanoparticles on MDR *S. pyogenes* by TEM

Nano *E. elaterium* effects on MDR *S. pyogenes* were determined using TEM pictures (JEOL JEM-1400 series TEM, Japan) that compared the morphology of *S. pyogenes* treated with *E. elaterium* nanoparticles to that of a control sample. Prior to adding 1mg of Nano *E. elaterium* and incubating at 37°C overnight, the bacterium was first inoculated into 2ml of nutrient broth. The mixture was centrifuged for 10 minutes. After the surplus was discarded, the palette was preserved in glutaraldehyde and osmium tetroxide and dehydrated in alcohol. After that, the test was coated with epoxy resin. The microtome slices' thickness was adjusted to be between 500 and 1000nm. Small pieces were stained with toluidine blue (1X) and viewed with a Leica ICC50 HD camera.

Comparing the cytotoxicity test of Nano *E. elaterium* to that of the medication gentamicin

Human gastric epithelial cell line (GESI) was obtained from the American Type Culture Collection and cultivated in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) (NY, USA), 10µg/ml of insulin (Sigma), and 1% penicillin-streptomycin (Sigma). For one day, 100µl of the tested material per well and complete growth medium were added to 96-well plates containing cell plates (Chen et al., 2018).

The initial enzymatic reduction modification of viability assay 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was used to quantify cytotoxicity in order to produce blue crystals known as formazan (Elkhateeb et al., 2024)

Cells were seen under an inverted microscope until the cell layer had spread after 2ml of trypsin EDTA solution had been added to the flask. Following the addition of 6ml of complete growth medium, the cells were gently pipetted out and centrifuged at 125 xg for five minutes. The cultures were kept at 37°C for a full day after the supernatant was disposed of and the cell pellet was suspended in new growth media. Prior to the MTT experiment, the plates were inspected under an elevated microscope.

The MTT method of measuring in vitro cytotoxicity performed well with multiwell plates. After reconstituting each vial of MTT [M-5655] with 3ml of media, 10% of the volume of the culture medium was added to the reconstituted MTT. MTT solubilization solution [M-8910] was added equivalent to the original culture medium to remove the formazan crystals that had formed after cultures had been back in the incubator for two hours, depending on the type of cell and maximum cell density. Different cell concentrations were cultured for 24 hours with *E. elaterium* nanoparticles and gentamicin drug (4.0, 16.0, 63.0, 250.0, and 1000.0µg/l) dissolved in 10% FBS. The plates were examined and absorbance at 450 nm was determined spectrophotometrically (BioTek Instruments, Inc. Winooski, VT, USA).

Statistical analysis

The experimental data were expressed using the mean ± standard error of the mean (SEM). Additionally, one-way ANOVA was performed to analyze the data gathered, and a significant difference was defined as one with a p-value of 0.05 or lower (Elshal et al., 2022).

RESULTS AND DISCUSSION

GAS continues to rank among the top ten infectious causes of mortality, contributing to a substantial global health burden, particularly in low- and middle-income nations (Auala et al., 2022).

Eighty isolates were found to be *S. pyogenes* in the current investigation. 22.50% were found in blood samples, while 77.50% were found in sputum samples. The age rate was classified as 18–25 (male 87.5% & female 12.5%), >25–35 (male 57% & female 43%), and >35–45 (male 56% & female 44%). Males were more affected by *S. pyogenes* than females, with rates of 66 and 34%, respectively. In each group, it is clear that males had a higher prevalence of *S. pyogenes* infection than females, may be because males tend to eat food outside the home compared to females. Elderly patients (>35–45Y) were more susceptible to *S. pyogenes* in clinical samples (47.5%) than other categorized as 18–25Y (20%) and >25–35Y (32.5%), may be because they are almost immune-compromised (Fig. 1). AL-Taei et al. (2016) carried out an opposite study. In the Baghdad Teaching Hospital, they discovered that *S. pyogenes* increased 3.07 percent from 260 samples, of which 75.8% came from blood and 24.2% from throat swabs. Similar to this finding, AL-Taei et al. (2016) showed that male patients (65.0%) had a greater frequency of pathogens identified from patients admitted to Baghdad than female patients (35.0%).

This result ran counter to that of Othman et al. (2019), who discovered that women had a statistically significant greater frequency *S. pyogenes* of GAS (66%) than men (34%). According to similar findings, women were exposed to infections at a higher rate than men (Anja et al., 2019).

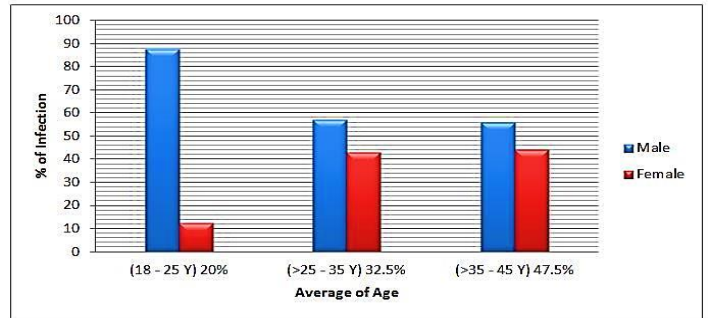


Figure 1 *S. pyogenes* prevalence in clinical samples in relation to patient age and gender.

The susceptibility of *S. pyogenes* strains to antibiotics was assessed. Of these, 22 out of 80 (27.5%) were identified as multidrug-resistant strains. Certain isolates of *S. pyogenes* shown resistance to the macrolides group (azithromycin 91.0%), cycline group (tetracycline 86.0%) and quinolones group (ciprofloxacin 82.0% and nalidixic acid 82.0%). On the other hand, susceptible to aminoglycoside group (gentamicin 100.0%), phenicol group (chloramphenicol 77.0%) and carbapenem group (meropenem 68.0%) (Fig. 2).

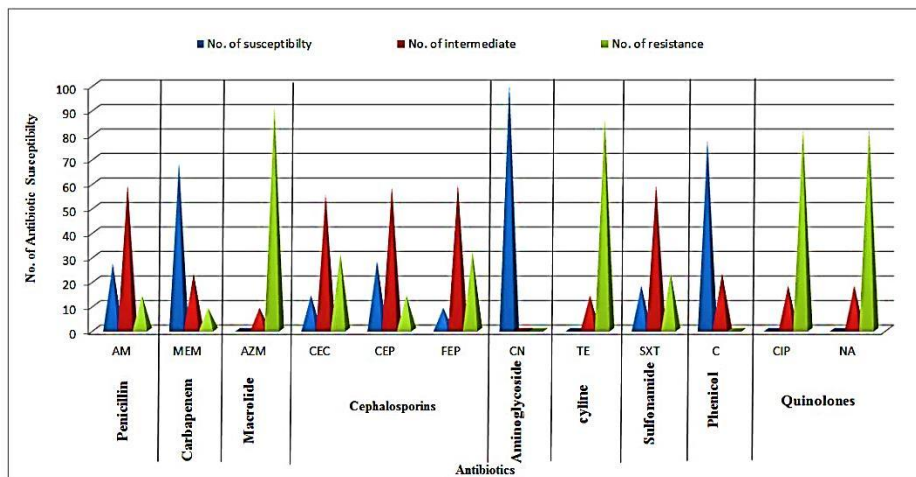


Figure 2 Percentages of multi-drug resistant *S. pyogenes* isolates on antibiotic discs.

According to several prior researches, their multidrug resistance (MDR) presents significant health risks. Some strains of *S. pyogenes* exhibit resistance to tetracycline, erythromycin, and cotrimoxazole (Khan et al., 2013). The proportion of co-resistance to quinolones and macrolides (10%) was alarming since new fluoroquinolones may be therapeutic alternatives for the treatment of infections caused by bacteria resistant to macrolides in patients with β -lactam allergies. Camara et al. (2013) produced the other discovery, finding that isolated *S. pyogenes* was completely resistant to tetracycline. According to Anja et al. (2019), tetracycline resistance is present in 42.9% of *S. pyogenes* isolates. Their multidrug resistance (MDR) trait has been linked to serious healthcare issues in previous research.

Genetic methods have a great potential in examination of strain involved in disease out breaks and in identification of the epidemic clones. Currently the standard in microbiological determination of these enzymes (Nordmann and Poirel, 2019)

The current study used PCR to identify the presence of the *mefA*, *parC*, *tetO*, and *tetM* genes that cause MDR *S. pyogenes*. The PCR product showed that nineteen strains out of 22 (86.36%) harbored *mefA* gene and seventeen strains out of 22 (77.27%) harbored *parC* gene. Seventeen strains out of 22 (77.27%) harbored *tetO* gene and 19 strains (86.36%) harbored *tetM* gene, whereas eight strains (36.36%) carried both four genes (Fig. 3).

Mef gene amplification was studied by Bley et al. (2011), who found that 31% of strains had the *mef* gene. A prior study that searched for genes linked to macrolide resistance found that only 26.1% of *S. pyogenes* strains displayed the *mef* gene (Katosova et al., 2016). According to Santoro et al. (2014), the presence of tetracycline resistance determinants (*tet* genes) in conjugative transposons, which may efficiently translocate across related bacteria, may account for the high occurrence of resistance. Giovanetti et al. (2003) discovered that 46 (73%) of the 63 strains of *S. pyogenes* that exhibited tetracycline resistance had the *tetO* gene.

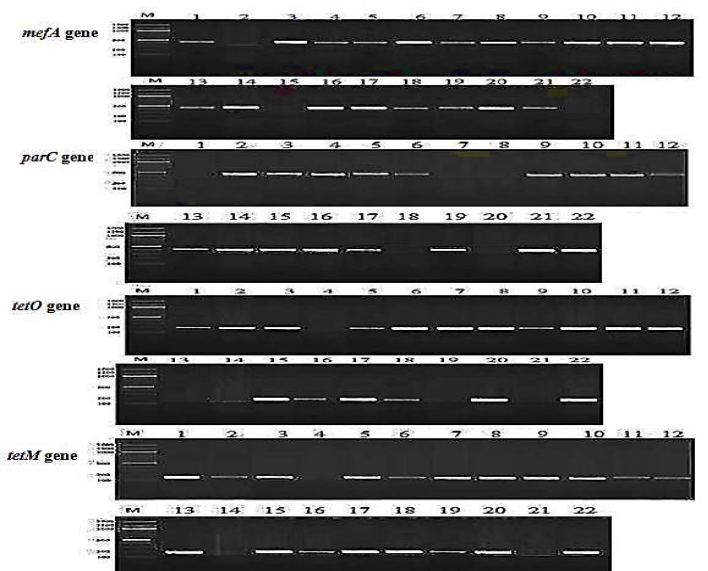


Figure 3 DNA segment of the *mefA* gene (432 bp), *parC* gene (429 bp), *tetO* gene (171 bp) and *tetM* gene (171 bp) with M as the marker and samples numbered 1, 2, 3,... to 22.

Antimicrobial compounds are common in medicinal plants. Due to their potential antibacterial action, a variety of medicinal plant extracts are utilized to treat a number of illnesses. As raw materials for several herbal businesses, some of these bioactive compounds are tested and marketed on the market (Renisheya et al., 2011).

In the current work, the filter paper disc technique was used to investigate the antibacterial activity of *E. elaterium* extract and nanoparticles on MDR *S. pyogenes* strains that included the resistance genes (*mefA*, *parC*, *tetO*, and *tetM*). *E. elaterium* nanoparticles' growth suppression zone on MDR *S. pyogenes* had a mean diameter of 20 mm, which was greater than that of the *E. elaterium* extract's (15 mm) (Fig. 4).

In a related study, **Elkhateeb et al. (2024)** used the well diffusion method to discover that *E. elaterium* extract and nanoparticles had antibacterial action against bacteria that are resistant to many drugs. According to **Muftah et al. (2013)**, *E. elaterium* had potent antioxidant properties. Due to the presence of certain phenolic compounds in the plant, *E. elaterium* "fruit juice" may have antibacterial and antioxidant properties that scavenge free radicals. According to **Adwan et al. (2011)**, the *E. elaterium* educator shown antimicrobial effectiveness against bacteria that are resistant to several drugs (15mm).

Different methods, such as DLS and TEM, are employed in the literature for the analysis of nanoparticles (**Arya et al., 2019**). In our study, A DLS was used to characterize the Nano *E. elaterium*. that showed their size was 33nm and using a TEM showed that their size was 18.1 nm, which is nearly similar to an investigation done by **Khan et al., (2023)**, who discovered that DLS showed the *E. elaterium* nanoparticle size range, the highest intensity was observed at size 99.36 nm. The TEM was used to examine the nanoparticles' shape, which showed that they had a smooth, spherical structure. The sizes of *E. elaterium* nanoparticles were also disclosed by TEM, with the smallest size being 17.8 nm.

The size estimated by TEM was less than the size acquired by DLS because DLS measures the hydrodynamic size of nanoparticles instead of their physical diameters (**Jang et al., 2015**). This indicates that as nanoparticles travel in a liquid medium, the solvent's electric dipole layer envelops them and may also affect how they move inside the solvent (**Alahmad et al., 2021**).

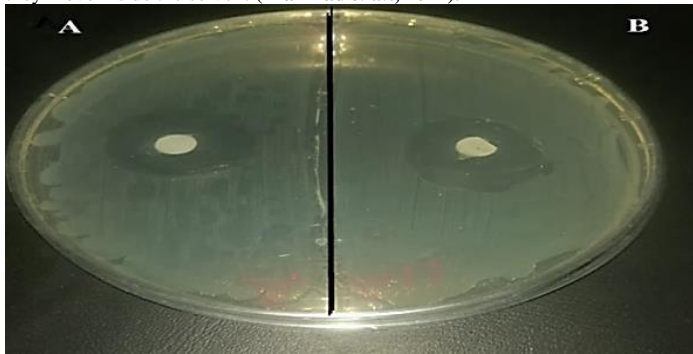


Figure 4 Agar disc diffusion technique bioassay of *E. elaterium* extract (A) (15 mm) and nanoparticles (B) (20 mm) on multidrug-resistant *S. pyogenes*.

In the present investigation, the MBC of nano *E. elaterium* was 30µg/ml and the MIC was 20µg/ml for MDR *S. pyogenes* that harboured the *mefA*, *parC*, *tetO* and *tetM* genes shown in table 2.

Table 3 Results of GES1 cells with Nano *E. elaterium* and Gentamicin drug

Specimen	Gentamicin/GES1					Nano <i>E. elaterium</i> /GES1				
Conc. of dilutions (µg/ml)	100	25	6.3	1.6	0.4	100	25	6.3	1.6	0.4
Mean	0.194	0.259	0.315	0.347	0.366	0.299	0.352	0.440	0.466	0.523
%	48.496	59.414	59.764	65.196	68.27	50.68	59.13	74.02	78.275	87.962
Cytotoxicity IC50	190.0±8µg/ml					1220.0±73µg/ml				

CONCLUSION

The current study's findings showed that *Ecballium elaterium* nanoparticles are highly effective against MDR *S. pyogenes* that had resistance genes for *mefA*, *parC*, *tetO*, and *tetM*. 20% was MIC and 30% was the MBC. We advise using natural products to treat multi-drug-resistant bacteria because gentamicin, the most effective drug of choice for MDR *S. pyogenes*, has a stronger inhibitory activity toward GES1 cells than Nano *E. elaterium*. As a result, Nano *E. elaterium* are less cytotoxic to GES1 normal cells than gentamicin drug.

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Conflict of Interest: The authors have no financial conflicts of interest to declare.

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In this study, TEM was used to assess the impact of Nano *E. elaterium* on multidrug-resistant *S. pyogenes* strains carrying four genes. Nuclear materials and cytoplasmic components were absent, and the cell membrane was disrupted (Fig. 5).

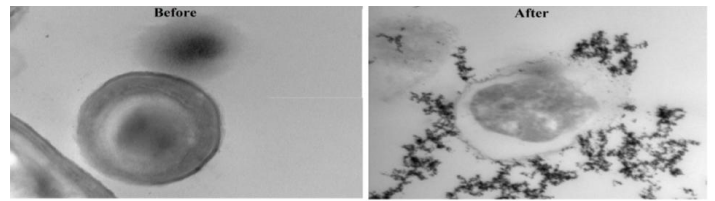


Figure 5 Image of TEM show MDR *S. pyogenes* comparing with after added *E. elaterium* nanoparticles.

Table 2 Different *E. elaterium* nanoparticle concentrations' antibacterial effects against MDR *S. pyogenes*

Conc. of <i>E. elaterium</i> nanoparticles (µg/ml)	Antibacterial activity of Nano <i>E. elaterium</i> against MDR <i>S. pyogenes</i> involves four genes.
1	0.689
10	0.650
20	0.620 (MIC)
30	0.790 (MBC)
40	0.951
50	0.994
60	0.998
70	1.031
80	1.055
90	1.077
100	1.098

Note: Positive control for antibacterial activity of *E. elaterium* nanoparticles **0.444nm**, Negative control for antibacterial activity of *E. elaterium* nanoparticles **1.388nm**

The original enzymatic reduction modification of the MTT viability test was used to quantify cytotoxicity for Nano *E. elaterium* in comparison to the most used antibiotic, gentamicin (10µg). These findings indicated that gentamicin has a stronger inhibitory action against GES1 cells than Nano *E. elaterium*. The IC50 is the concentrations of an inhibitor where the response is reduced by half *E. elaterium* nanoparticles is 1220.0±73µg/ml and gentamicin drug is 190.0±8µg/ml shown in table 3. These results were shown that Nano *E. elaterium* are less cytotoxic to GES1 cells than gentamicin drug.

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