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Research article

Quantitative analysis and antimicrobial efficacy of the ethanol extract obtained from *Durio graveolens* leaves

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Abstract

Objectives: This study aims to explore the natural antimicrobial properties of ethanol extracts from *Durio graveolens* leaves (DGL). **Methods:** DGL underwent preliminary phytochemical screening, along with a quantitative assessment of phenols and flavonoids. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. Antibacterial activity against Gram-positive and Gram-negative bacteria was evaluated using the agar well diffusion method. The microorganisms tested included ATCC strains of *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, and *Escherichia coli*. **Results:** DGL demonstrated the highest MIC of 0.1 mg/ml and an MBC of 0.25 mg/ml against the Gram-negative bacteria *P. aeruginosa* and *E. coli*. At an MIC of 0.1 mg/ml, DGL produced significant zones of inhibition against *P. aeruginosa* and *E. coli* when compared to gentamicin. **Conclusion:** This research indicates that DGL possesses natural antibacterial properties against Gram-negative human pathogens.

Introduction

Infections from microorganisms, including bacteria, fungi, and viruses, present a serious threat to patients, especially those with weakened immune systems. These infectious diseases are major contributors to morbidity and mortality in this population [1, 2]. For many years, antimicrobials have been used effectively to treat infections, leading to improved health outcomes. However, the rise of antimicrobial resistance has become a pressing global issue, impacting public health and hindering a country's economic and social progress [3-6].

The misuse of antimicrobials in treating infections is a primary factor driving resistance. Moreover, the food industry plays a role by promoting the selection of resistant microorganisms throughout the food supply chain[5]. The heavy use of antimicrobial agents in animal husbandry has significantly contributed to resistance development. Ongoing genetic exchanges among microorganisms have created a reservoir of resistance genes, which can lead to new forms of resistance [6].

Bacteria that carry resistance genes or can thrive despite exposure to multiple antibiotics are classified as multidrug-resistant bacteria [1]. Consequently, there is an urgent need for new and more effective antimicrobial agents that can either inhibit the growth of microorganisms (microbiostatic) or eliminate them (microbicidal). Plant-based antimicrobial substances offer considerable benefits, as they are often effective in addressing these challenges and generally have fewer side effects compared to synthetic antimicrobials [4].

Leaves are the most commonly utilized parts of the plant, typically prepared by boiling in water (decoction) and taken orally. Common health issues treated with these medicinal plants include stomach problems, respiratory conditions, wounds, and muscle pain or fatigue in women [3]. *Durio graveolens*, belonging to the Bombacaceae family, is native to Peninsular Malaysia, where its distinctive characteristics support widespread cultivation [2]. Research has indicated that the bioactive compounds found in durian include anthocyanins, flavonoids, carotenoids, and flavanols in its pulp, with antioxidant and antiproliferative levels varying based on ripeness. Nearly all parts of the durian, including the fruit, pulp, leaves, and roots, are traditionally used to treat various ailments [7, 8]. The leaves of *Durio graveolens* (DGL) have been traditionally utilized by the Kensie tribe in Kedah, Malaysia, as a remedy for fever and influenza by incorporating the macerated leaves into bathing water [9, 10]. This study aims to qualitatively and quantitatively analyze the presence of various phytochemical constituents and assess the antimicrobial potential of *D. graveolens* leaves against common human pathogens.

Methods

Sample collection

Fresh and uninfested leaves of *Durio graveolens* were collected from Baling, 09100, Kedah, Malaysia. These samples were identified and recorded with a herbarium voucher specimen accession number (AIMST/FOP/03).

Preparation of extract

The collected *Durio graveolens* leaves were washed in running water and sun-dried for at least 2 days to prevent chemical decomposition. Once dried, the leaves were ground using a Waring Blender. Extraction was carried out using a percolation method with a Soxhlet apparatus and a single solvent system consisting of 70% ethanol. The leaves were measured at 200 g, sliced into small pieces, and prepared for hot percolation. This process continued until the solvent's green color nearly disappeared in the Soxhlet tube. The extract was then filtered through a muslin cloth to separate the residue from the filtrate. The residue was adjusted to the desired concentration, and the extraction was repeated until a clear, colorless supernatant was obtained. Finally, the filtrate was evaporated using a rotary evaporator [11].

Qualitative phytochemical screening

Preliminary phytochemical screening of the ethanol extract from *Durio graveolens* was performed to identify various phytoconstituents, including gum, anthocyanins, starch, monosaccharides, reducing sugars, carbohydrates, amino acids, proteins, tannins, glycosides, phenols, mucilage, steroids, alkaloids, flavonoids, and saponins, using standard biochemical methods [8].

Quantitative phytochemical screening determination of total phenolic content

The total phenolic content in the extract was assessed using a modified spectrophotometric method [10, 12]. Samples were prepared at concentrations between 0.005 mg/ml and 0.02 mg/ml. The reaction mixture contained the sample (100µl), Folin-Ciocalteu's reagent (100µl), and 2.5% sodium carbonate solution (2 ml), which was incubated at room temperature for 30 minutes. Absorbance was measured at 750 nm. The procedure was repeated with standard Gallic acid (GA) to develop a calibration curve. The phenolic concentration was expressed as GA equivalent in mg/g (GA mg/g) based on the absorbance readings.

Determination of total flavonoid content

The flavonoid content of the extract was determined using a modified spectrophotometric method [13, 14]. Samples were prepared in concentrations ranging from 0.01 mg/ml to 0.1 mg/ml. The reaction included the sample (1 ml), 30% ethanol (10 ml), and 10% aluminum chloride solution (0.7 ml), which was left to incubate at room temperature for 6 minutes. Afterward, 10 ml of 1 M sodium hydroxide solution was added, and the final volume was adjusted to 25 ml with 30% ethanol, allowing it to rest for 10 minutes. Absorbance was measured at 450 nm. This process was repeated with standard Rutin (RU) to create a calibration curve. The flavonoid concentration was expressed as RU equivalent in mg/g (RU mg/g) based on the absorbance values.

antibacterial activity Mcfarland 0.5 standard

The standard was created by mixing 0.5 ml of a 1.175% barium chloride solution with 99.5 ml of 1% sulfuric acid. The tubes were sealed and stored in the dark at room temperature. This standard produces turbidity (OD = 7) that closely matches that of a bacterial suspension containing 1.5 × 10^8 colony-forming units (CFU)/ml or 1.5 × 10^5 CFU/µl. The turbidity of the inoculum suspensions was compared by observing the black lines through the liquid [16].

Microorganism selection and collection

The selection of microorganisms such as Bacillus subtilis, Enterococcus faecalis, Staphylococcus aureus, Streptococcus pyogenes, Neisseria gonorrhoeae, Pseudomonas aeruginosa, and Escherichia coli is based on their diverse roles in biotechnology, medicine, and industrial applications:

Bacillus subtilis: Known for its ability to produce enzymes and probiotics, it is widely used in fermentation and as a model organism in research.

Enterococcus faecalis: This bacterium is important in the study of antibiotic resistance and can be used in probiotic formulations due to its beneficial properties.

Staphylococcus aureus: A key pathogen, it serves as a model for studying pathogenicity, antibiotic resistance, and biofilm formation.

Streptococcus pyogenes: Known for its role in human infections, it is crucial for researching virulence factors and developing vaccines.
Neisseria gonorrhoeae: This pathogen is selected for its significance in public health and studies on antibiotic resistance and treatment efficacy.
Pseudomonas aeruginosa: A model organism for studying biofilm formation and antibiotic resistance, it is also relevant in environmental microbiology.
Escherichia coli: A widely used model organism in molecular biology and genetics, it is essential for recombinant DNA technology and protein production.
These microorganisms were chosen to provide a comprehensive understanding of both beneficial and pathogenic properties, facilitating research across various fields.
Bacillus subtilis (ATCC 6051): A Gram-positive, rod-shaped bacterium known for its ability to form spores and produce enzymes. Used in biotechnology for enzyme production.
Enterococcus faecalis (ATCC 29212): A Gram-positive cocci, common in the human gut, known for its role in antibiotic resistance.
Staphylococcus aureus (ATCC 25923): A Gram-positive cocci, a major human pathogen causing a variety of infections.
Streptococcus pyogenes (ATCC 19615): A Gram-positive cocci responsible for diseases like strep throat and skin infections.
Neisseria gonorrhoeae (ATCC 19424): A Gram-negative diplococcus, the causative agent of gonorrhea, significant for studying sexually transmitted infections.
Pseudomonas aeruginosa (ATCC 27853): A versatile Gram-negative bacillus known for its resistance to antibiotics and its role in nosocomial infections.
Escherichia coli (ATCC 25922): A Gram-negative rod, a model organism for genetic studies and a common inhabitant of the human gut.

Collection sites for microbes

Bacillus subtilis: Often isolated from soil samples, particularly in agricultural regions.
Enterococcus faecalis: Collected from human fecal samples or gut flora.
Staphylococcus aureus: Found in nasal passages of healthy individuals or from infected wounds.
Streptococcus pyogenes: Isolated from throat swabs of infected patients.
Neisseria gonorrhoeae: Collected from clinical samples of infected individuals (urethral, cervical swabs).
Pseudomonas aeruginosa: Isolated from soil, water, or clinical samples from infected patients.
Escherichia coli: Commonly found in fecal samples or environmental samples from water sources.

determination of minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

The MIC was determined using a test tube dilution method. One ml of nutrient broth was added to sterile test tubes. Then, 1 ml of a 1 mg/ml test solution was transferred to one tube and serially diluted to concentrations ranging from 0.03125 mg/ml to 0.5 mg/ml. Following this, 0.1 ml of the inoculum was added, and the tubes were incubated at 37°C for 24 hours. Growth in the tubes was assessed visually for turbidity. The MIC was defined as the lowest concentration of extract that inhibited bacterial growth, indicated by the absence of turbidity in the broth [15].
The MBC was determined by comparing the number of viable bacteria in the inoculum to the initial count. All tubes from the MIC test that exhibited no visible turbidity were serially diluted and spread onto nutrient agar plates for viable cell counting (CFU). These plates were incubated at 37°C for 24 hours. The MBC was recorded as the lowest concentration of extract that killed at least 99.99% of the initial bacterial count [12].

Agar well diffusion method

The antibacterial activity was assessed using the agar well diffusion assay [13]. The extracts were evaluated for their antibacterial effects against three Gram-negative bacteria (*Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, and *Escherichia coli*) and four Gram-positive bacteria (*Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Streptococcus pyogenes*). Bacterial inocula were evenly spread on Müller-Hinton agar plates using a sterile cotton swab. A volume of 50 µl of each extract was added to four wells (12 mm diameter, spaced 20 mm apart) in the agar. The plates were incubated for 24 hours at 36°C ± 1°C under aerobic conditions. After incubation, bacterial growth was examined, and the inhibition of growth was measured in millimeters. Gentamicin was used as a positive control, while 10% dimethyl sulfoxide served as a negative control [18].

Results

The findings from the phytochemical screening, as detailed in Table 1, demonstrate the presence of a variety of phytoconstituents within the extracts of *Durio graveolens*. These constituents include glycosides, mucilage, steroids, carbohydrates, flavonoids, alkaloids, and phenols, indicating a rich phytochemical profile.
To quantify the phenolic content of the extract, measurements were taken using the standard Gallic acid (GA) calibration curve, which is depicted in Figures 1a and 1b. The analysis revealed a strong correlation ($r^2 = 0.8104$) between the concentration of phenolic compounds and their absorbance, as illustrated by the linear regression equation (RE) expressed as $y = 0.0032x + 0.044$. Based on these measurements, the total phenolic content found in the leaves

of *Durio graveolens* (referred to as DGL) was calculated to be 1.80 mg per 100 g of crude extract, as shown in Figure 1.

In addition to phenolic compounds, the flavonoid content present in the *Durio graveolens* extract was also assessed. This was accomplished by using a standard Rutin (RU) calibration curve, illustrated in Figures 2a and 2b. The analysis yielded a linear regression equation of $y = 0.0004x + 0.0067$, with a high degree of correlation ($r^2 = 0.9723$) observed between the concentrations of flavonoids and their corresponding absorbance values. Consequently, the total flavonoid content in the *Durio graveolens* leaves was determined to be 1.34 mg per 100 g of crude extract, as represented in Figure 2.

The antimicrobial activity of *Durio graveolens* leaves (DGL) was assessed against four Gram-positive and three Gram-negative ATCC bacterial strains. The findings presented in Table 2 indicate the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of DGL. The highest MIC recorded was 0.1 mg/ml, while the MBC was 0.25 mg/ml for the Gram-negative bacteria *P. aeruginosa* and *E. coli*. At an MIC of 0.1 mg/ml, as shown in Table 3, *Durio graveolens* produced notable zones of inhibition against *P. aeruginosa* (11.2 ± 0.76 mm) and *E. coli* (12.0 ± 1.00 mm), in comparison to gentamicin, which showed inhibition zones of 12.3 ± 1.15 mm and 13.7 ± 0.58 mm, respectively (Figure 3). The extract exhibited minimal antimicrobial activity against *S. aureus* and *N. gonorrhoeae*. All results are expressed as mean ± standard deviation (n=3).

Discussion

Medicinal plants are recognized for their therapeutic properties, which stem from the phytoconstituents that trigger notable pharmacological actions in humans [18]. These plants serve as an essential source of numerous antimicrobial compounds. Several phytochemical constituents, including polyphenols, flavonoids, phenolics, tannins, terpenoids, and sesquiterpenes, are identified as effective antimicrobial agents against a wide variety of microorganisms. This study indicated that *Durio graveolens* leaves (DGL) are a significant source of antimicrobial agents effective against Gram-negative bacteria, especially when compared to those targeting Gram-positive bacteria. The array of phytoconstituents present, particularly flavonoids, phenols, and alkaloids, may play a crucial role in the antibacterial efficacy observed.

The antimicrobial activity of the extract is likely linked to its elevated flavonoid content, which has been associated with the inhibition of nucleic acid synthesis and other metabolic pathways [21]. Flavonoids have also been documented to prevent the germination of spores in plant pathogens [17]. Certain phenolic compounds, especially those with a C3 side chain at low oxidation levels and without oxygen, have been found to contribute to antimicrobial effects.

Additionally, the partial hydrophobic nature of phenolic compounds is another factor in their antimicrobial properties. The toxic mechanisms of polyphenols against pathogens might involve the inhibition of hydrolytic enzymes, particularly proteases, or various interactions that disrupt microbial adhesion, cell envelope transport proteins, and non-specific interactions with carbohydrates [19].

Table 1. Qualitative phytochemical analysis of leaves of *Durio graveolens*.

Phytochemical Constituents	DGL (Durio graveolens leaves Ethanolic extract)
Alkaloids	Present (+)
Amino Acids	Absent (-)
Anthocyanins	Absent (-)
Carbohydrates	Present (+)
Flavonoids	Present (+)
Glycosides	Present (+)
Gums	Absent (-)
Mucilage	Present (+)
Monosaccharides	Absent (+)
Phenols	Present (+)
Proteins	Absent (-)
Reducing Sugar	Present (+)
Saponins	Absent (-)
Starch	Absent (+)
Steroids	Present (+)
Tannins	Absent (-)

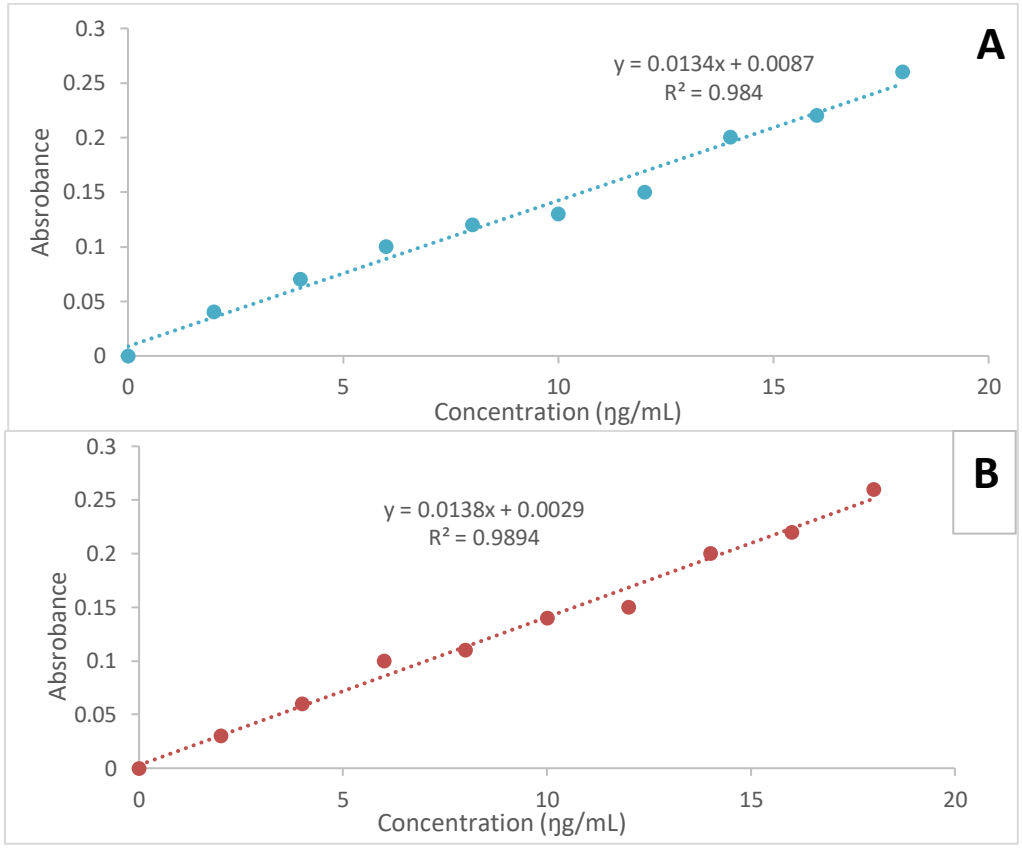

Figure 1. Standard calibration curve of both gallic acid (A) and the leaves of *Durio graveolens* ethanol extracts (B).

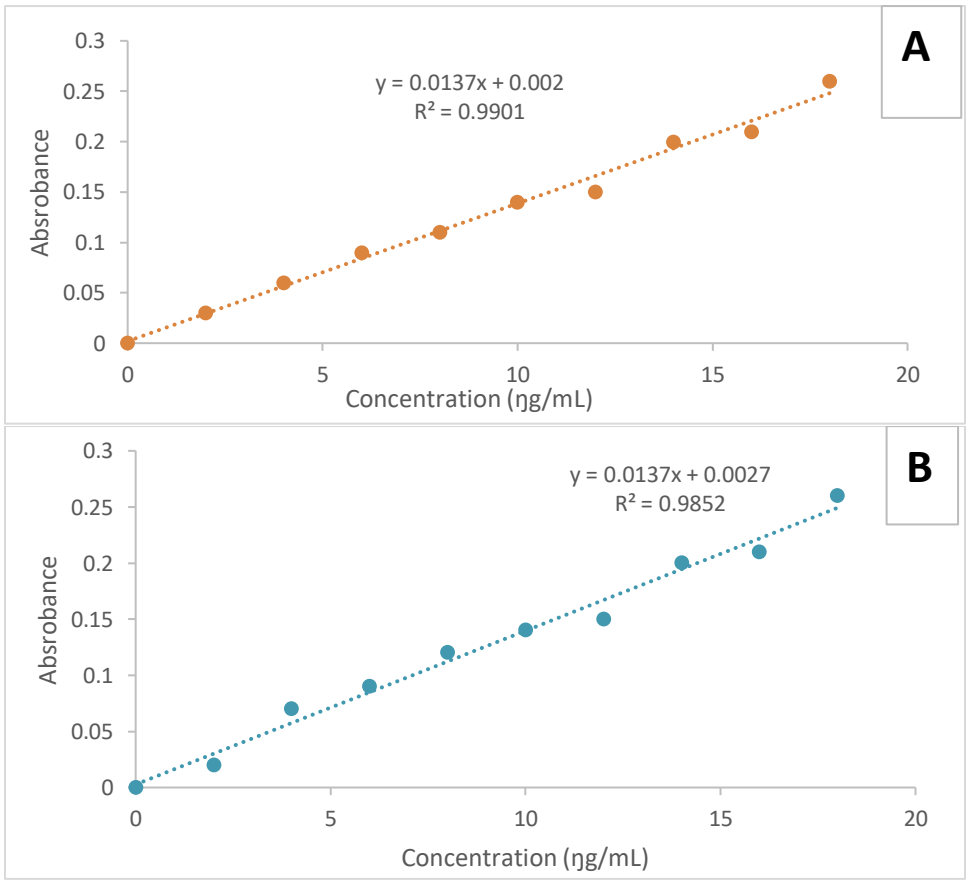

Figure 2. Standard calibration curve of rutin (A) and the leaves of *Durio graveolens* ethanol extracts (B).

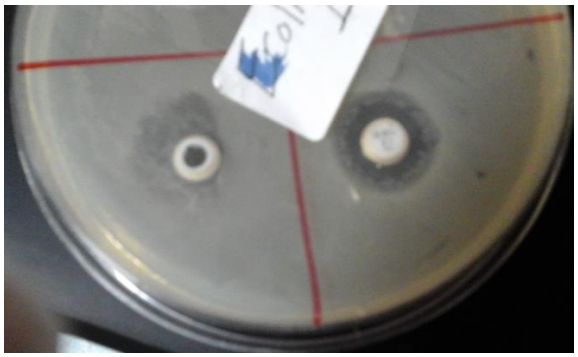

Figure 3. Zone of inhibition of Ethanolic extract of Durio graveolens leaves against Escherichia coli.

Table 2. The results of MIC and MBC of ethanolic extract of *Durio graveolens* leaves.

Microorganisms	Ethanolic extract of *Durio graveolens* leaves	MIC (mg/mL)	MBC (mg/mL)
Bacillus subtilis (ATCC 11774)	Yes	0.15	0.6
Enterococcus faecalis (ATCC 29212)	Yes	0.30	1.2
Escherichia coli (ATCC 10799)	Yes	0.12	0.3
Neisseria gonorrhoeae (ATCC 43069)	Yes	0.08	0.2
Pseudomonas aeruginosa (ATCC 10145)	Yes	0.09	0.22
Staphylococcus aureus (ATCC 29213)	Yes	0.14	0.55
Streptococcus pyogenes (ATCC 19615)	Yes	0.13	0.45

MIC: Minimum inhibitory concentration, and MBC: Minimum bactericidal concentration.

Table 3. The zone of inhibition of Ethanolic extracts of *Durio graveolens* leaves in comparison Gentamicin as positive control.

Microorganisms	Zone of Inhibition of Ethanolic extracts of *Durio graveolens* leaves (mm, 0.25 mg/mL) ± SD	Gentamicin inhibition zone (mm, 0.25 mg/mL) ± SD (positive control)
Bacillus subtilis	8.0 ± 0.60	14.0 ± 1.00
Enterococcus faecalis	8.2 ± 1.10	14.3 ± 0.58
Escherichia coli	12.5 ± 0.90	13.7 ± 0.58
Neisseria gonorrhoeae	6.0 ± 0.60	14.3 ± 1.15
Pseudomonas aeruginosa	11.5 ± 0.80	12.3 ± 1.15
Staphylococcus aureus	4.0 ± 1.10	14.0 ± 1.00
Streptococcus pyogenes	8.0 ± 0.55	10.7 ± 0.58

Conclusion

Our research indicates that *Durio graveolens* leaves (DGL) contain important phytoconstituents that are linked to their antimicrobial effects. The extract shows the ability to combat various human pathogens, likely due to its significant flavonoid and phenolic content. However, further investigation is required to isolate and characterize the active compounds in *Durio graveolens*, as well as to understand their biological mechanisms, to facilitate the development of effective antimicrobial drugs.

Ethics Approval and Consent to Participate

Ethical approval is not applicable to this study as there were no human participants involved. The research was conducted in accordance with applicable institutional guidelines.

Consent for Publication

All authors have provided their consent for publication and affirm that they approve the final version of the manuscript.

Conflicts of Interest

The authors declare that there are no conflicts of interest related to this publication. All financial and personal relationships that could potentially influence the study have been disclosed.

Author Contribution

All authors are contributed equally in the research

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