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IN-SILICO AND *IN-VITRO* BACTERICIDAL ACTIVITY OF THE PHYTOCHEMICALS OF *PEPEROMIA PELLUCIDA* (L.) HERB

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Background: *The development and spread of pathogenic antibiotic resistance to many antibiotics have been growing worldwide. This concept is of enormous importance, and it is crucial to identify appropriate therapeutic drugs to fight against these antibiotic-resistant microbes. Plant species can make a valuable contribution to new antibiotics to replace or improve existing treatment potential.* ***Methods:*** *In this work, we conducted a molecular modelling study of three phytochemicals (Peperomin A, Peperomin E, Peperomin F) with bacterial targets penicillin-binding protein (2C6W) from Streptococcus pneumoniae and kdpFABC complex (5MRW) of Escherichia coli. Peperomia pellucida methanolic extract was conducted antimicrobial activity against clinical Streptococcus pneumoniae and Escherichia coli.* ***Results:*** *Three phytochemicals (Peperomin A, Peperomin E, Peperomin F) showed the best docking results with target antimicrobial-resistant targets. Peperomia pellucida methanolic extract showed good antibacterial activity against clinical Streptococcus pneumoniae and Escherichia coli.* ***Conclusion:*** *In-vitro and In silico bactericidal studies conducted, Peperomia pellucida phytochemical has significance antibacterial activity against clinical Streptococcus pneumoniae, Escherichia coli.*

INTRODUCTION

In India, traditional medicines are commonly used by all groups of Indian either directly as natural medicines or selectively in pharmaceutical formulations for natural drugs[1]. In India alone, 2000 various species are used for medicinal preparations for both externally and internally use, as per National Health Experts. It is almost challenging to combat infectious and non-infectious diseases, particularly those due to multidrug-resistant microorganisms such as *Staphylococcus*, *Escherichia coli*, *Enterococcus* species and reactive oxygen species[2]. Novel treatments for successful public health care merit pathogens' resistant rate to vast synthetic antimicrobial agents coupled with growing side effects of antibiotics. Accordingly, several studies on the bioactive phytochemical constituents of essential oils, alkaloids, polyphenols, and flavonoids have presented as potential choices in recent years[3].

The use of medicinal herbs increases worldwide, with various people using these phytoconstituents to overcome several national healthcare sceneries[4].

Four billion people live in developing countries depend on herbal medicines as their primary source of health care. Natural medicines treatment seen as an important cultural tradition in these societies[5&6].

Peperomia pellucida (shiny bush, silver bush) of the Piperaceae family is an annual herbaceous plant. The monsoon season grows to a height of 15 – 46 cm in wet, loose soil, particularly under trees. *Peperomia pellucida* commonly found in West African rainforest belts, Southeast, Southwest Nigeria, and many tropical Asian and South American

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countries[7&8]. *Peperomia pellucida* ethno-medicine reports indicate that the leaf's usage varies depending on the area where it is found[9]. It has used to lower cholesterol levels, such as diuretic, dementia, and cardiac arrhythmia. In Ayurvedic records, the aqueous mixture of leaves and stem used to manage bleeding, fever, headache, abdominal pain, wound dressing and cough suppression[10]. The *Peperomia pellucida* whole plant decoction India's used as a potent medication for rheumatism, renal disorders, breast cancer, boils, and smallpox[11&12]. Previous pharmacological studies have shown that solvents' raw extracts display significant analgesic, antimicrobial, anti-inflammatory, anti-protozoal and cytotoxic activity in the cell line of breast cancer. The crude solvent extracts suggested by the presence of alkaloids, sterols, flavonoids and styrene as dominant bioactive compounds of *Peperomia pellucida*[13]. We aimed for this research analysis in silico and *In-vitro* bactericidal activity of the bioactive molecules *Peperomia pellucida* (L.) herb.

METHODS

Chemicals and Media

The reagents and chemical used have included the following: Nutrient broth, Muller-Hinton agar**,** Muller-Hinton broth**,** Ethanol**,** Phenol**,** Sterile discs**,** Methanolic extract. All the chemicals used in this study were analytical standard.

Collection, identification of plant and preparation of crude extract

P. pellucida was collected in August 2020 from Kerala India at the Patambi (Fig. 1). Plant taxonomist (Dr Dhanapal V principal and professor at sri sastha college of pharmacy Chennai, India) authenticated the plant. The entire plant was left air-dry at an ambient room temperature for 15 days. Air-dried plant pulverised, and the each 100 (gms) of the pulverised plant were extracted with methanol using a modified Clevenger-type apparatus for 5 hrs. The mechanical-assisted extraction to obtain active compounds for bioactivity assays.

Fig. 1: *Peperomia pellucida.*

Study of antibacterial activity

Antibacterial activity by well plate method

The bactericidal activity of crude methanolic extract (*Peperomia pellucida*) and standard drug (Amikacin-100mg/2ml) was studied using a well plate method. *Streptococcus pneumoniae*, *Escherichia coli* Inoculum, have been made using the media of the broth culture. Double strength sterile MHA (Mueller Hinton agar) media was prepared by autoclaving of 7.6 gm in 100 ml. Inoculate the test bacteria on the MHA plates with sterile cotton swabs. Crude methanolic extract (100 mg) dissolved in DMSO solvent and 100µl cured extract and Amikacin (10µg) were positioned on agar well. Plates incubated in the freezer for 30 min to spread the extraction into the agar plate and, ultimately, set at 37°C for 24 hrs. Antibacterial activity assessed using the Himedia zone reader[14&15].

MIC and Minimum bactericidal concentration

The serial dilution method used to study the bactericidal activity of the crude methanolic extract (DMSO dissolved) and respective controls. A spectrophotometer (OD595= 0.22) equivalent to 10^8 CFU/mL used to fix the clinical bacterial cultures (*Streptococcus pneumoniae*, *Escherichia coli*) to 0.22 optical density at 595 nm. DMSO dissolved crude methanolic extract, respectively added in 2.0 mL MIC tubes and 10µL of the inoculum. The bacterial culture was then serially diluted. These MIC tubes were incubated overnight at 37°C, followed by a viable CFU bacteria count[16].

Statistical analysis

The values expressed as mean ± standard deviation and the ANOVA's statistical comparisons followed by Duncan's Multiple Range Test using Windows SPSS version 12.0. The values considered statistically significant if the p-value is less than 0.05.

Docking study

To have a clearer idea of the inhibitory mechanism and interaction of the raw extract pharmacogenetic substances *P. pellucida* (Peperomin A, Peperomin E, Peperomin F), docking analysis accomplished using the Autodocking vina. Phytoconstituent (Peperomin A, Peperomin E, Peperomin F) selected for the study based on the literature review. The protein prepared for docking by removing water, adding polar hydrogens and Kollman charges. Understand the protein-ligand interactions through Discovery studio visualiser. Two primary drug-target-pathways, i.e., penicillin-binding protein (2C6W) from *Streptococcus pneumoniae* and kdpFABC complex (5MRW) of *Escherichia coli* were subject to estimate the mechanism of phytoconstituents (Peperomin A, Peperomin E, Peperomin F). Chemical structures (Fig. 2) retrieved from the PubChem. Binding pockets for the target protein identified using the CASTp server[17&18].

The antibacterial activity conducted triplicate and its produced significant ($p< 0.05$) zone of inhibition against *Streptococcus pneumoniae*, *Escherichia coli*. The results of the antibacterial activity of *P. pellucida* methanolic extract showed in table 1 and figure 3.

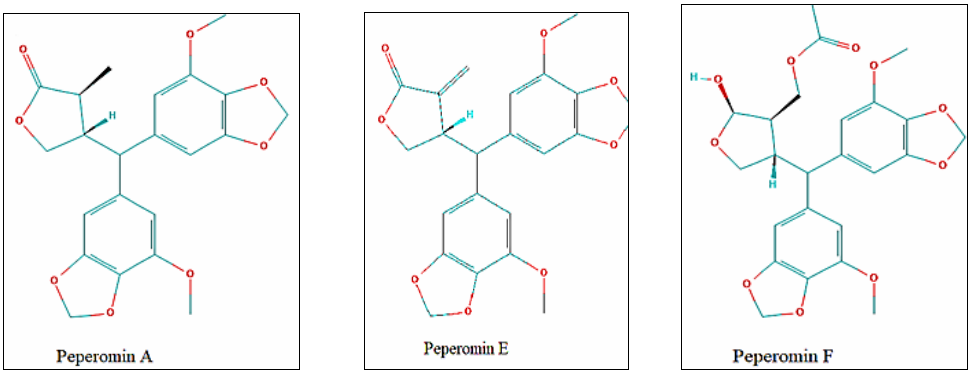


Fig. 2: 2D structures of Peperomin A, E, F (*P. pellucida* Phytochemicals).

Table 1: Bactericidal activity of methanolic extract and standard drug against clinical *Streptococcus pneumoniae*, *Escherichia coli*.

S.NO	Formulations / Standard drug	Zone of inhibition (mm)	
		Streptococcus pneumoniae (*n*= 2)	*Escherichia coli* (*n* =2)
1	Amikacin 100 µl (50 µg)	14±5 mm	25±5 mm
2	Methanolic extract (DMSO Solvent) (2000 µg)	16±5 mm	15±5 mm

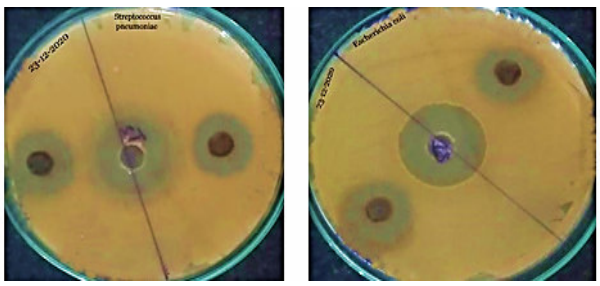


Fig. 3: Antibacterial activity of methanolic extract and standard drug against clinical *Streptococcus pneumoniae*, *Escherichia coli.*

RESULTS AND DISCUSSION

Results

In-vitro bactericidal activity

Agar well diffusion method

DMSO dissolved methanolic extract's bactericidal activity initially determined by well diffusion against the clinical bacteria *Streptococcus pneumoniae*, *Escherichia coli*. The *P. pellucida* methanolic extract's bactericidal activity showed that the mean inhibition zone (16±5 and 15± mm).

MIC (minimum inhibitory concentration) and minimum bacterial concentration

Crude methanolic (DMSO dissolved) extract efficacy was tested against clinical *Streptococcus pneumoniae*, *Escherichia coli* and calculating the MIC and the minimum bacterial concentration. MIC and minimum bacterial concentration values obtained from the methanolic extract were 2000 µg/mL, 1000 µg/mL against clinical *Streptococcus pneumoniae*, *Escherichia coli* (Tables 2 and 3).

Docking studies

Show the *in-silico* study results considered appropriate to carry out molecular docking studies that compare both in silicon and *in-vitro*. Docking studies have seen at different stages of the investigation, such as predicting ligand-receptor interactions and detecting compounds based on binding energies. In this study, the docking of the tested compounds with the primary drug pathway for Streptococcus pneumoniae, Escherichia coli and the corresponding fitness score was also determined, as shown in table 4. Interactive forces showed their highest interaction affinity.

In the docking studies of peperomin A, E and F the molecular binding pattern has revealed that it has hydrogen, carbon-hydrogen, alkyl, pi-donor and pi-pi stacked bonds 2C6W (penicillin-binding protein – PBP-*Streptococcus pneumoniae*).

Table 2: Target MICs µg/mL for clinical pathogenic microorganisms.

S.NO	Formulations/Standard drug	MIC	MBC
		Streptococcus pneumoniae (*n* =2)	
1	Amikacin (500-3.90625)	62.5	31.25
2	Methanolic extract (4000-µg)	2000	1000

Showing activity against clinical pathogenic *Streptococcus pneumoniae*.

Table 3: Target MICs µg/mL for clinical pathogenic microorganisms (*Escherichia coli*).

S.NO	Formulations/Standard drug	MIC	MBC
		Escherichia coli (*n*= 2)	
1	Amikacin (500-3.90625)	62.5	31.25
2	Methanolic extract (4000-µg)	2000	1000

Showing activity against clinical pathogenic *Escherichia coli*.

Table 4: Docking score of 2C6W, 5MRW with ligands.

Protein	Ligand	Binding energy (kcal/mol)
2C6W	Peperomin A	-6.7
	Peperomin E	-8.4
	Peperomin F	-6.6
5MRW	Peperomin A	-7.7
	Peperomin E	-7.8
	Peperomin F	-9.1

Peperomin A energy observed to be -6.7 Kcal/mol against 2C6W. Conventional hydrogen (SER 462, 466), carbon-hydrogen (ASN 562), pi-donor hydrogen (TYR 409, 563) and alkyl (TYR 401) bonds observed (Fig. 4 (A)).

Peperomin E energy observed to be -8.4 Kcal/mol against 2C6W. Conventional hydrogen (SER 428), carbon-hydrogen (ASN 430), pi-sigma (TYP 411), pi-pi stacked (TRP 321) and alkyl (PHE 577) bonds were observed (Fig. 4 (B)).

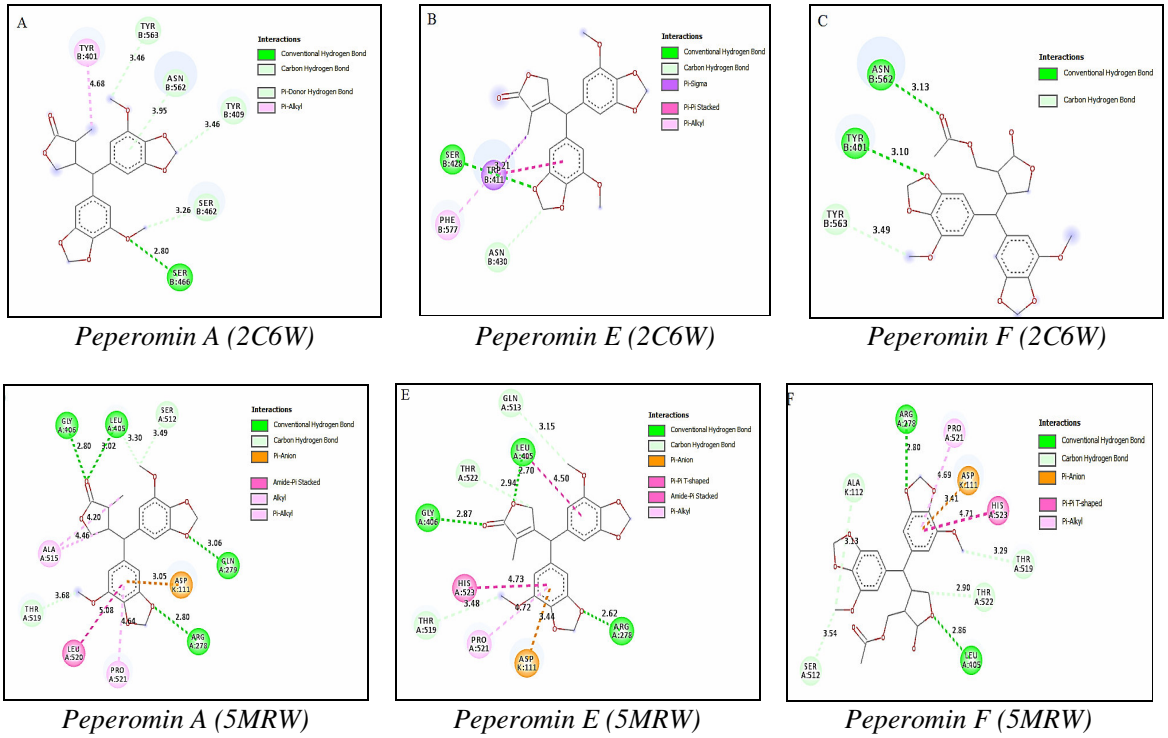
Peperomin F energy observed to be -6.6 Kcal/mol against 2C6W. Conventional hydrogen (ASN 562, TYR 401) and carbon-hydrogen (ASN 430) bonds were observed (Fig. 4 (C)).

The docking studies of peperomin A, E and F molecular binding pattern revealed that it has hydrogen, carbon-hydrogen, alkyl, pi-Anion, Amide-pi-Stacked, Pi-alkyl and alkyl bonds with 5MRW (KdpFABC complex - hydrolase - *Escherichia coli*).

Peperomin A energy detected to be -7.7 Kcal/mol against 5MRW. Conventional hydrogen (ARG 278, GLU 279, LEU 405 and GLY 406), carbon-hydrogen (SER 512, THR 519), pi-Anion (ASP 111), Amide-pi-Stacked (LEU 520), Pi-alkyl (ALA 515) and alkyl (PRO 521) bonds observed (Fig. 4 (D)).

Peperomin E energy observed to be -7.8 Kcal/mol against 5MRW. Conventional hydrogen (ARG 278, GLU 279, LEU 405 and GLY 406), carbon-hydrogen (THR 519, THR 522, GLN 513), pi-Anion (ASP 111), pi-pi T shaped (HIS 523), Amide-pi-Stacked (HIS 523) and Pi-alkyl (PRO 521) bonds observed (Fig. 4 (E)).

Peperomin E energy observed to be -9.1 Kcal/mol against 5MRW (Hydrolase). Conventional hydrogen (ARG 278 and LEU 405), carbon-hydrogen (ALA 112, THR 519, THR 522, SER 512), pi-Anion (ASP 111), pi-pi T shaped (HIS 523) and Pi-alkyl (PRO 521) bonds observed (Fig. 4 (F)).



Peperomin A (2C6W) *Peperomin E (2C6W)* *Peperomin F (2C6W)*

Peperomin A (5MRW) *Peperomin E (5MRW)* *Peperomin F (5MRW)*

Fig. 4: 2D Interactions with 2C6W (A) Peperomin A, (B) Peperomin E, (C) Peperomin F.
2D Interactions with 5MRW. (D) Peperomin A, (E) Peperomin E, (F) Peperomin F.

In this study, Peperomin A, E, F showing interaction with the 2C6W and 6MRW signifying antibacterial activity. *P. pellucida* annual herb with Peperomin A, E and F compounds shows significant antibacterial activity against clinical pathogenic bacteria *Streptococcus pneumoniae*, *Escherichia coli*.

Conclusion

Peperomia pellucida crude methanolic extract and phytoconstituents (Peperomina A, E and F) have bactericidal activity against *Streptococcus pneumoniae*, *Escherichia coli*. In Silico studies, molecular binding interactions data have shown that Peperomine A, E and F are bound to the penicillin-binding protein (PBP) and KdpFABC complex on hydrolase binding site. However, several scientific studies have shown that lead drug development schemes on the origin of binding efficacy indices can provide active molecules with better pharmacokinetic performance. Identified bioactive compounds used to create more reasonable structural activity interactions in the era of bactericidal drug development.

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نشرة العلوم الصيدلية
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المواد الكيميائية النباتية لعشب البيبروميا بيلوسيدا كمضاد الجراثيم حاسوبيا وفي المختبر خارج الخلية

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الخلفية: إن تطور وانتشار مقاومة الميكروبات للعديد من المضادات الحيوية آخذ في الازدياد في جميع أنحاء العالم. هذا المفهوم له أهمية كبيرة ، لتحديد الأدوية العلاجية المناسبة لمحاربة هذه الميكروبات المقاومة للمضادات الحيوية. النباتات تستطيع ان تساهم في تقديم المضادات الحيوية الجديدة لتحل محل أو تحسن إمكانات العلاج الحالية.

الطرق: في هذا العمل ، أجرينا دراسة حاسوبية لثلاث مواد كيميائية نباتية (بيبرومين ا وبيبرومين ي وبيبرومين ف) بأهداف بكتيرية بروتين رابط للبنسلين (2C6W) من المكورات العقدية الرئوية السريرية و kdpFABC complex (5MRW) من الإشريكية القولونية. تم دراسة نشاط مستخلص الميثانول لعشب البيبروميا بيلوسيدا كمضادًا للميكروبات ضد المكورات العقدية الرئوية السريرية والإشريكية القولونية.

النتائج: أظهرت ثلاث مواد كيميائية نباتية (بيبرومين ا وبيبرومين ي وبيبرومين ف) أفضل نتائج في الدراسة الحاسوبية في مقاومة مضادات الميكروبات. أظهر المستخلص الميثانولي لعشب البيبروميا بيلوسيدا نشاطًا مضادًا للبكتيريا جيدًا ضد المكورات العقدية الرئوية السريرية وايشرشيا كولي.

الإستنتاج: الدراسات التي أجريت حاسوبيا وفي المختبر خارج الخلية كمضاد الجراثيم ، البيبرميا بيلوسيدا مادة كيميائية نباتية لها نشاط هام مضاد للجراثيم ضد البكتيريا العقدية الرئوية السريرية ، الإشريكية القولونية.