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TO STUDY THE ANTIBACTERIAL ACTIVITY OF *PSIDIUM GUAJAVA* EXTRACT IN MULTI DRUG RESISTANT GRAM NEGATIVE UROPATHOGENS AT A TERTIARY CARE HOSPITAL KANPUR, UTTAR PRADESH.

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ABSTRACT

INTRODUCTION: *Psidium guajava*, is a plant with known antimicrobial properties. The current study was carried out to investigate the efficacy of leaves extract of *Psidium guajava* as an alternative source of antimicrobial substance or an active biomolecule against Uropathogens. **AIM:** To study the Antibacterial Activity of *Psidium guajava* extract in Multi Drug Resistant Gram negative Uropathogens at a tertiary care Hospital Kanpur, Uttar Pradesh. **MATERIAL & METHODS:** This was a cross-sectional study conducted in the Department of Microbiology, for a period of 12 months, from November 2023 - November 2024 at Rama Medical College Hospital and Research Centre Kanpur. A total of 45 sample were cultured and antimicrobial susceptibility was performed according to CLSI guidelines 2024. The *Psidium guajava* plant leaves were collected from the medical garden of RMCH&RC. The antimicrobial activity of *Psidium guajava* was studied by kirby-Baur disc diffusion and well diffusion method. **RESULTS:**Out of 45 urine samples, **16** samples were found E. coli, sensitive to Polymyxin-B, Colistin and Tigecycline. Whereas, **8** samples were found Pseudomonas aeruginosa, sensitive to Polymyxin, Colistin . They are found to be Multi Drug Resistant.3 samples were found Staphylococcus aureus, were sensitive to Amikacin, Linezolid , Teicoplanin , Vancomycin. They are found to be methicillin-Resistant Staphylococcus aureus(MRSA). The antibacterial activity of each extract (Ethanol, Methanol, Acetone, Distilled Water) was evaluated using agar well diffusion & disc diffusion, and it was observed that antibacterial effects of ethanolic extracts of guava leaves against uropathogens, Pseudomonas aeruginosa (29± 0.3mm), S. Aureus (19± 0.3mm) , and least for E.coli (16± 0.3mm). *Psidium guajava* ethanolic extract showed the higher antimicrobial activities. **CONCLUSION:** This study demonstrates the antimicrobial potential of *Psidium guajava* leaves extract by using various solvents. The results of antimicrobial susceptibility test by Kirby-Bauer disc diffusion, indicate that ethanol is better for the extraction of the antibacterial properties of guava leaves. **KEYWORDS:** *Psidium guajava,* kirby-Baur, Disc Diffusion, CLSI, Antimicrobial susceptibility test.

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INTRODUCTION

We are currently living in the future predicted by Alexander Fleming, where there is greater access to antibiotics [1] and the rampant use of the same in animal husbandry and fisheries [2]. The problem of antibiotic resistance appears to be more complex when associated with common infections like urinary tract infection (UTI) [3].

The rising drug resistance urges the need for new drug development programs. However, with the current scenario, it is evident that the new drugs formulated after years of research and huge expenditure will eventually become ineffective [4].

The emerging problem of antibiotic resistance has driven an urge among scientists to explore alternative treatment methods by pursuing the teachings of traditional medicine. It is for this reason that recently the focus of researchers are shifted towards the use of natural products obtained from herbal sources to be used as antimicrobial agents.

Psidium guajava, commonly known as guava, is one such plant with known medicinal properties [5]. It belongs to the *Myrtaceae* family that also consists of plants like clove, allspice, and eucalyptus whose medicinal properties are well documented [6].

Traditionally, the leaves of this plant have been useful in overcoming stomach ailments like gastroenteritis, vomiting, diarrhoea and dysentery [7]. The essential oils extracted from the leaves of this plant show presence of tannins, terpenes, flavonoids and resin, which together contribute to the antimicrobial properties reported in various literature [8]. In addition to the antimicrobial properties, they also exhibit regulating effects on lifestyle disorders like diabetes, hypertension, and obesity [9].

The problem of antibiotic resistance appears to be more complex when associated with common infections like urinary tract infection (UTI) [10]. The β-lactams are among the most frequently prescribed antibiotics for UTIs due to its efficacy, broad-spectrum activity and low toxicity [11]. Over the years, the indiscriminate use of these antibiotics has consequently resulted in selective pressures among pathogens, generating mutations in the β-lactamase genes which produce resistance that is far more resilient to treatment by current antibiotics. The ESBL producing pathogens are resistant to third generation cephalosporins e.g., cefotaxime, ceftazidime, ceftriaxone etc. In addition to these antibiotics, MBL producers show resistance to last resort carbapenem antibiotics viz., meropenem, ertapenem and imipenem [12].

Since UTIs are the second most common infections occurring worldwide, the occurrence of ESBL and MBL genes among uropathogens is especially troublesome because of its associated risk of uncontrolled and easy dissemination in the environment.

Considering the increasing antibiotic resistance among pathogens, Will be investigating the antibacterial as well as resistance reversal activity of leaves extract of *Psidium guajava* against ESBL, MBL producing uropathogens.

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MATERIALS AND METHODS

Preparation of plant extract

- The leaves of *Psidium guajava* will be washed with distilled water, dried in shade for 8 to 10 days, and ground to fine powder with the help of a mechanical blender.
- The extraction of bioactive compounds will be carried out, from a 100g sample in 200mL ethanol using Soxhlet apparatus, over a period of 12 hours.
- The extract will be further concentrated at 40°C on a water bath to obtain a semisolid mass. This mass was re-suspended in ethanol to get the required concentration of the extract for carrying out further analysis.
- These concentrates will be prepared in large volumes and stored at 4°C until further use, in order to avoid batch to batch variations in our study.
- Water extract of *P. guajava* will also be prepared by boiling the leaves in distilled water for 15mins.
- The sterility of the extracts would be confirmed by checking for bacterial or fungal growth after spot inoculating them on a sterile Nutrient Agar (NA) and Sabouraud's Agar plate respectively. The NA plates were incubated at 37°C and SAB plates at 30°C for an extended duration of 7 days to confirm the absence of contaminants.

TEST PROCEDURE

Qualitative study of the inhibitory activity of *P. guajava* ethanolic leaves extract against test organisms

- The antibacterial effect of *P. guajava* ethanolic leaves extract against test pathogens will be determined by agar well diffusion method [17].
- Sterile molten NA butt would be seeded with 0.4ml of 24h old test pathogens (0.1 OD_{540nm}) and poured into sterile petri-plates. After solidification, wells were punched into the medium using a sterile cork-borer and 50µl of plant extracts were added to the same.
- Then it would be allowed to diffuse through the wells during its incubation at 37°C for 24h, after which the resulting zones of inhibition were measured. Control wells would also set up using 50µl of ethanol (solvent) for each isolate.

Determination of MBC of *P. guajava* ethanolic leaves extract against test pathogens

- Agar dilution method will be carried out to determine the Minimum Bactericidal Concentration (MBC) of *P. guajava* ethanolic leaves extract against test pathogens.
- Different concentrations of ethanol extract ranging from 5mg/mL to 50 mg/mL with an interval of 5 mg/mL will be supplemented into molten NA butts cooled to around 40^0C.

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- The test isolates will be spot inoculated (5µL) on solidified medium and incubated at 37°C for 24h. MBC would be defined as the lowest concentration of *P. guajava* ethanolic leaves extract that completely inhibited the growth of test cultures.

Evaluation of Synergistic effect by agar dilution method

- The agar dilution method will be similarly used to determine the synergistic activity between *P. guajava* leaves extract and ampicillin.
- It would be carried out by incorporating sub-lethal (½MBC) concentrations of *P. guajava* leaves extract into molten NA butt which were cooled to around 40°C along with 100-500 µg/mL of ampicillin with an interval of 100µg/mL.

Gas Chromatography-Mass Spectrophotometry analysis

- The bioactive components from *P. guajava* leaves extract will be analysed by GC-MS HP 7890 system (Agilent technologies).
- Capillary column with dimensions 30m X 0.25mm X 0.25µm will be equipped. The program used for GC oven temperature will be 5min isothermal at 300ºC, followed by 90º-260ºC at a rate of 10ºC/min, then held at 260ºC for 5 min.
- The injection port temperature will be 240ºC. Along with that a Joel, AccuTOF GCV MS system, with a time-of-flight analyser, was used.
- The compounds of the *P. guajava* leaves extract will be identified by comparison of their retention indices (RI) and mass spectra fragmentation with those on the stored library available with CDRI Lucknow.

RESULTS

Out of 45 urine samples, **16** samples were found E. coli. They were sensitive to Polymyxin-B (13-19 mm), Colistin (11-15 mm) and Tigecycline (20-27 mm). They are found to be Multi Drug Resistant. Whereas, **8** samples were found Pseudomonas aeruginosa. They were sensitive to Polymyxin-B (14-18 mm), Colistin (11-15 mm) and Tigecycline (9-13 mm) They are found to be Multi Drug Resistant.**2** samples were found Staphylococcus aureus. They were sensitive to Amikacin (20-26mm), Linezolid (25-32mm), Teicoplanin (15-21mm), Vancomycin (17-21mm). They are found to be methicillin-Resistant Staphylococcus aureus (MRSA). And **1** sample was found to be Micrococci.

The antibacterial activity of each extract (Ethanol, Methanol, Acetone, Distilled Water) was evaluated using agar well diffusion & disc diffusion, and it was observed that antibacterial effects of ethanolic extracts of guava leaves against uropathogens i.e. S. Aureus, Pseudomonas aeruginosa, Micrococci and E. Coli, showed the highest zone of inhibition against Micrococci (35± 0.3mm) followed by Pseudomonas aeruginosa (29± 0.3mm), S. Aureus (19± 0.3mm) , and least for E.coli (16± 0.3mm).

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On comparing the antimicrobial efficacy of sensitive drugs and *Psidium guajava* ethanolic extract it was observed that ethanolic leaves extract shows the higher antimicrobial activities.

The qualitative analysis (GCMS) of ethanolic extract of *Psidium guajava* leaves contain tannin, flavonoids, steroids, terpenoids, carbohydrate, polyphenol, glycoside etc.

DISCUSSION

The present study investigated the antimicrobial activity of Psidium guajava leaf extracts; the results showed that the ethanolic extracts of guava leaf inhibited the growth of the Gram Negative Uropathogens tested. These results support the findings of Viera et al. (2001), Egharevba et al. (2010) and Biwas et al. (2013) which also reported the antibacterial effect of guava leaves extracts and found that they inhibited the growth of Uropathogens. However, the ethanolic extract showed stronger inhibition than the aqueous extract against the organisms. This result is in conformity with that of Pandey, (2012) who reported that the antibacterial activity ethanolic extract of Psidium guajava leaf and stem showed stronger anti-bacterial activity than aqueous extract. The present result is contrast with the findings of (Emmanuel, 2010 and Biwas et al., 2013) who reported higher antimicrobial activity of aqueous extract of Psidium guajava than that of ethanolic extract. The result of the preliminary phytochemical analysis of leaf extract, (ethanol and water) of P. guajava revealed the presence of the following chemical constituents; Alkaloid, saponin, phenol, flavonoids, protein and amino acid, anthraquinones, terpenoid and tannin.

Therefore, the results of this study justifies that the phytochemical solvents of leaf extracts of P. guajava possess antibacterial properties that could inhabit microorganisms as well as believed to contribute in a way as humans continue to source for total cure for infectious diseases especially with the growing trends of antimicrobial resistance.

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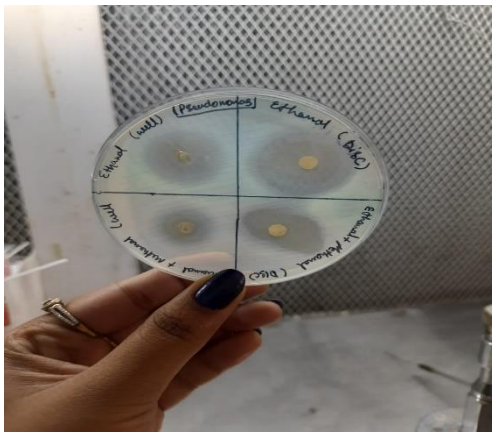

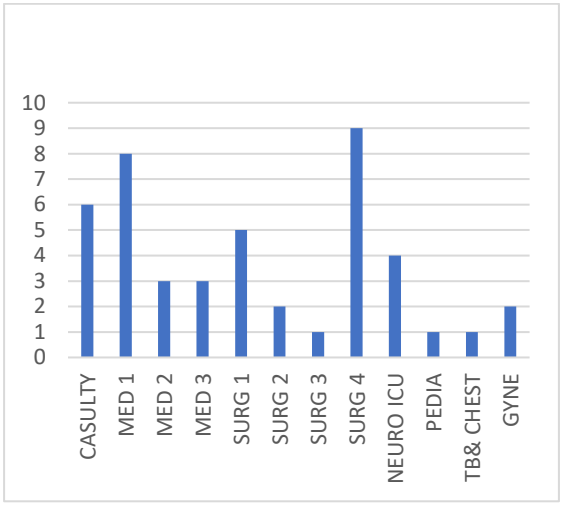

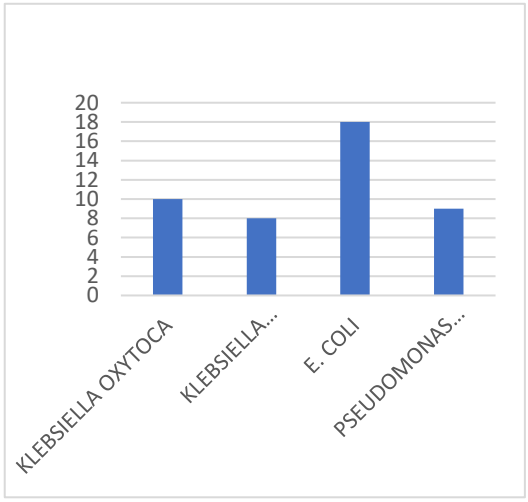

CONCLUSION

The ethanolic extract of leaves showed significant P. guajava activity against multidrug resistant gram negative uropathogens. Further, its potency can be evaluated against pathogenic drug-resistant bacteria causing other infections. Moreover, the ability of crude extracts to give such promising antibacterial results only enhances the chances of it becoming an important alternate remedy towards the treatment of infections caused by multi- drug resistant pathogens. To increase its use, additional research is required on its degree of toxicity and any potential antagonistic or synergistic interactions with other plants or medications

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