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A pilot study on antimicrobial activity of *Psidium guajava* leaf extracts against clinical species of *Klebsiella pneumoniae*

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Abstract

Background and Objective: Recurrent asymptomatic urinary tract infections caused by *Klebsiella pneumoniae* highlight the need for alternative treatments due to increasing antimicrobial resistance. This study aimed to isolate *Klebsiella pneumoniae* and evaluate the potential of *Psidium guajava* leaf extracts as a natural treatment for these infections.

Materials and Methods: Aqueous extracts of *Psidium guajava* were prepared at concentrations of 0.025, 0.05, 0.1, and 0.2 mg/ml from both dried and fresh leaves. Mid-stream urine samples were collected from presumed healthy individuals. Microbiological and biochemical analyses were conducted on the samples. Antimicrobial activity was assessed using the agar well diffusion method, with *Klebsiella pneumoniae* ATCC 13883 serving as the reference strain.

Results and Conclusion: Aqueous extraction at higher concentrations enhanced antimicrobial activity. Cold water extracts of fresh leaves showed better inhibition at higher concentrations (total range of 0-10 mm for four extracts), while hot water extracts inhibited *Klebsiella pneumoniae* in the range of 4-10 mm. Dried leaf extracts were more potent, with inhibition zones of 7–17 mm for cold water and 10-20 mm for hot water, demonstrating the superior efficacy of dried leaves over fresh. *Psidium guajava* leaf extracts demonstrate antimicrobial potential against *Klebsiella pneumoniae*, suggesting a promising alternative for urinary tract infections. Further trials are needed to confirm safety, efficacy, and mechanisms of action, offering potential treatment options where conventional antibiotics fail.

Keywords: Antibacterial efficacy, Antimicrobial resistance, *Klebsiella pneumoniae*, *Psidium guajava*, Urinary tract infections

1. Introduction

Urinary tract infection (UTI) is a common bacterial infection that affects any part of the urinary system, including the kidneys, ureters, bladder, and urethra. It occurs when bacteria enter the urinary tract and multiply, leading to irritation and infection [1]. *Escherichia coli* is responsible for over 80% of community-acquired UTI cases [2]. Other bacteria such as Klebsiella, Enterococcus, and *Staphylococcus saprophyticus* can also cause UTIs [2]. UTIs are

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among the most common bacterial infections worldwide, affecting millions of people annually. Women are more prone to UTIs due to their shorter urethras, which allow easier access for bacteria to reach the bladder [3]. However, men and children can also develop UTIs, often associated with underlying health conditions [4].

Sexual activity, poor hygiene, catheter use, suppressed immune systems, and underlying medical conditions increase susceptibility to UTIs [3]. Several other factors increase the likelihood of UTIs, including female anatomy, use of certain contraceptives (such as spermicides), a history of UTIs, urinary tract abnormalities, and conditions like diabetes [3]. Socioeconomic factors, such as limited access to healthcare, poor hygiene practices, and lack of education about UTI prevention, can contribute to increased UTI prevalence in certain populations [5]. Personal behaviors, like inadequate fluid intake, not emptying the bladder completely, or holding urine for extended periods, can also heighten the risk of developing UTIs [6]. UTIs can range from mildly uncomfortable to severe and potentially life-threatening if left untreated. Early recognition of symptoms, proper hygiene, and timely treatment play crucial roles in managing UTIs and preventing complications.

Preventive measures include adequate hydration, proper hygiene (wiping front to back, urinating after sexual activity), avoiding irritating substances like certain feminine products or spermicides, and seeking prompt treatment for urinary symptoms [7]. Vaccines and alternative therapies are also areas of ongoing research for UTI prevention [3].

Antibiotics are the primary treatment for UTIs. However, the rise of antibiotic resistance poses a significant challenge [8]. Overuse or misuse of antibiotics contributes to this issue, making certain bacteria less responsive to treatment [9]. As a result, healthcare providers seek effective treatment while being mindful of antibiotic stewardship to combat resistance.

The escalating challenge of antibiotic resistance in *Klebsiella pneumoniae* strains isolated from UTIs necessitates urgent exploration of alternative treatment avenues. With Klebsiella's propensity for multidrug resistance, identifying novel therapies is critical. Therefore, discovering potent alternatives, such as natural extracts, holds significant clinical relevance, potentially offering safer and more effective treatments while curbing the burden of antibiotic resistance in UTIs. *Psidium guajava* leaves contain bioactive compounds known for their antibacterial properties, making them a promising candidate for combating antibiotic-resistant strains. Hence, this study aims to explore the antimicrobial efficacy of extracts from *P. guajava* leaves against clinical isolates of *K. pneumoniae*, one of the main species inducing UTIs.

2. Materials and Methods

2.1. Location of study

The study was conducted in Wukari, a town in Southern Taraba, North-East Nigeria. Wukari is a key area known for its cultural significance, diverse population, and economic activities. As part of Southern Taraba, Wukari shares the region's rich heritage, ethnic diversity, and reliance on agriculture as a primary economic activity. The town's historical importance, demographic landscape, and development initiatives make it an ideal location for research within the broader context of Southern Taraba's cultural and economic dynamics.

2.2. Collection and identification of *P. guajava* leaves

Fresh leaves of *P. guajava* were collected in Wukari and identified by a plant expert at Federal University Wukari. Fresh leaves were immediately air-dried under a shade for eight days and immediately processed for further analysis.

2.3. Preparation of aqueous extract of *P. guajava* leaves

Approximately, 70 g of dried *P. guajava* leaves were weighed and soaked in 750 ml of distilled water for 72 h for both cold and hot extracts, and agitated until a deep color was achieved. The extract was separated from the leaves by filtering through filter paper and a

funnel into a sterile container. The obtained extract was evaporated. Concentrations of 0.025, 0.05, 0.1, and 0.2 mg/ml were prepared using sterile water and stored in well-corked sterile universal bottles.

2.4. Collection of urine samples

A sterile universal container was used to collect early morning mid-stream urine samples (specimen) from non-hospitalised volunteers who were within the ages of 18-30 years in Wukari. The samples were labelled appropriately with volunteers' data.

2.5. Isolation of *K. pneumoniae* clinical strains

Urine samples were inoculated onto MacConkey agar plates as soon as they were brought to the laboratory, following the standard procedure [10]. First, the surfaces of the MacConkey agar plates were dried before inoculating the urine samples. A sterilized wire loop was used to collect a loopful of the urine sample, which was applied to a small area on the plates. The sample was spread on the surface of the plates by streaking. This was done near a burner flame to prevent contamination. The inoculated plates were labeled appropriately and incubated at 37 °C for 24 h. After incubation, all suspected growth, which appeared pinkish and mucoid along the line of streaking, was picked and sub-cultured onto MacConkey agar plates. The MacConkey agar plates were labeled and incubated at 37 °C for 24 h to obtain discrete colonies of *K. pneumoniae*. Isolates were further subjected to biochemical analysis. A reference strain of *K. pneumoniae* ATCC 13883 was used to ensure the accuracy and reliability of this study.

2.6. Antimicrobial susceptibility test

Using the pour plate method, 1 ml of the suspension from the identified *K. pneumoniae* was added to sterile plates. The prepared nutrient agar was poured into each plate, rocked, and mixed homogeneously with the test organism, then allowed to solidify. A sterile cork-borer was used to make five wells in the plates (four wells for extracts at concentrations of 0.025, 0.05, 0.1, and 0.2 mg/ml, with one well designated for sterile water as a negative control). These plates were labeled appropriately. Then, 0.1 ml of each extract concentration and control was added to the wells using a sterile syringe. The plates were incubated further at 37 °C for 24-48 h to investigate the zones of inhibition.

3. Results and Discussion

Table 1 represents the biochemical and morphological characteristics of *K. pneumoniae*. The observed results confirmed that the isolates were *K. pneumoniae*.

The antimicrobial effects of fresh and dried *P. guajava* leaf extracts on *K. pneumoniae* clinical isolate were evaluated using both cold water and hot water extraction methods.

Table 1- Biochemical and morphological characteristics of the clinical isolates

Culture media	Morphological characteristic	Gram stain	Biochemical test						
			CAT	GAS	MR	LAC	SUC	GLU	URE
MacConkey agar	Pink or reddish mucoid colonies with a pink coloration, often displaying a characteristic metallic sheen.	Negative bacilli	+	+	+	+	+	+	+
Nutrient agar	Appear as smooth, convex, and round with a cream to a light-yellow colour.								

* CAT: catalase; GAS: gas production; MR: methyl red; LAC: lactose fermentation; SUC: sucrose fermentation; GLU: glucose fermentation; URE: urease

Table 2 shows that hot water extraction consistently exhibited greater antimicrobial activity compared to cold water extraction for fresh leaves. Across all concentrations tested, hot water extracts consistently produced larger zones of inhibition, ranging from 4 to 10 mm, compared to cold water extracts, which ranged from 0 to 10 mm. Furthermore, as the concentration of the extract increased, there was a proportional increase in the size of the inhibition zones for both extraction methods. Similarly, hot water extracts of dried leaves consistently resulted in larger zones of inhibition compared to cold water extracts of dried leaves (Table 3). Across all concentrations tested, hot water extracts consistently exhibited greater antimicrobial activity, with inhibition zones ranging from 10 to 20 mm, compared to cold water extracts which ranged from 7 to 17 mm. According to Table 2, as the concentration of the extract increased, there was a proportional increase in the size of the inhibition zones for both extraction methods.

Table 2- Inhibition zones (mm) of fresh *P. guajava* leave extracts against *K. pneumoniae* isolate

Concentration (mg/ml)	Cold water extracts	Hot water extracts
0.025	No inhibition	4
0.05	5	5
0.1	7	8
0.2	10	10

Table 3- Inhibition zones (mm) of dried *P. guajava* leave extracts against *K. pneumoniae* isolate

Concentration (mg/ml)	Cold water extracts	Hot water extracts
0.025	7	10
0.05	11	13
0.1	14	16
0.2	17	20

This outcome aligns with other studies that discovered the antimicrobial activities of *P. guajava* against gastroenteric and urinary tract pathogens [11,12]. The antimicrobial activity exhibited by *P. guajava* against *K. pneumoniae* is due to its bioactive contents, including flavonoids, quercetin, kaempferol, phenolic acids, and carotenoids [13].

The enhanced efficacy of the dry leaf extract compared to the fresh leaf extract was attributed to the higher moisture content of the latter, which fosters microbial survival and growth [14]. Furthermore, the moisture content of fresh leaves can alter the concentration of phytochemicals compared to dried leaves [15]. However, drying processes can lead to the degradation or loss of certain heat-sensitive compounds, potentially affecting the overall phytochemical profile [16]. Nonetheless, drying certainly concentrates the phytochemicals by removing water, leading to a higher proportion of bioactive compounds in the dried leaves compared to their fresh counterparts [17]. This concentration effect can potentially enhance the potency or efficacy of certain phytochemicals in dried leaves. For instance, flavonoids, phenolic compounds, and some antioxidants might become more concentrated in dried leaves due to water removal. However, certain phytochemicals, especially those sensitive to heat or oxidation, may degrade during the drying process, leading to a reduction in their content compared to fresh leaves [18]. This was evident in the study by Vidinamo et al. [19], indicating that some vitamins and enzymes can be degraded or lost through heat exposure during drying, impacting the nutritional and functional properties of the dried leaves. Additionally, the specific drying method employed can influence the phytochemical content. Air-drying, sun-drying, or dehydration at different temperatures and durations can differently affect the preservation

and concentration of phytochemicals [20]. In our study, air-drying was used as it is more effective in minimizing heat exposure and maintaining optimal conditions for retaining a higher content of phytochemicals in dried leaves compared to other methods [21].

The insignificant zones of inhibition at lower concentrations, especially for cold water extract, suggest an initially limited antibacterial effect. This similarity at lower concentrations implies a threshold below which antimicrobial properties might not be pronounced. The notable divergence at higher concentrations, especially for hot water extracts, echoes findings from a previous study [14], suggesting restrained effects at lower concentrations. The disparity in outcomes between cold and hot water extracts at higher concentrations might originate from differences in extract composition or the solubility of bioactive compounds. Plausibly, the hot water extraction facilitated the release of a greater quantity or more potent array of antimicrobial constituents from *P. guajava* compared to the cold-water method, resulting in increased antibacterial effects observed. This was consistent with the findings of Moongngarm et al. [21], which found that pressurized hot water better extracted phytochemicals from plants than cold water. Indeed, cold water extraction might not effectively solubilize certain key antibacterial compounds present in *P. guajava* at lower concentrations, resulting in limited antibacterial effects [22]. This suggests that the compounds responsible for antibacterial activity might require higher concentrations or specific conditions to be adequately extracted in cold water. Conversely, the hot water extraction method, especially for chemicals at higher concentrations, might facilitate the release and solubilization of a broader spectrum of bioactive compounds with higher antibacterial potential [21]. Heat can aid in breaking down cell walls or structures within plant material, allowing for greater extraction of compounds that might possess stronger antibacterial properties [23]. The observed variation could also be attributed to the nature of the bioactive compounds. Some antibacterial agents might be more readily soluble in hot water compared to cold water, resulting in enhanced antibacterial activity observed in the hot water extracts at higher concentrations [24].

The choice of using water-based extraction in this study aligns with the aim of universal health coverage, emphasizing cost-effectiveness and safety, mirroring traditional healers' preference for water due to its accessibility in preparing medicines from various sources. In summary, the study demonstrates that both hot water extraction and higher concentration levels contribute to acceptable antimicrobial effects of *P. guajava* leaf extracts against *K. pneumoniae* clinical isolates. These findings underscore the importance of extraction method, concentration level, and leaf state in optimizing the antimicrobial efficacy of *P. guajava* leaf extracts for potential therapeutic applications.

4. Conclusion

The experimental investigation into the antibacterial potential of *P. guajava* extracts revealed intriguing distinctions influenced by extraction methods and concentration levels. Cold water extracts consistently exhibited lower antibacterial effects, especially at lower concentrations, than hot water extracts. Similarly, with limited antimicrobial activity on the test organism at lower concentrations, dried leaf extracts displayed an escalating trend of antibacterial effectiveness with higher concentrations, indicating a potential for increased potency. This study enriches existing literatures by illuminating the intricate interplay between extract concentration, extraction methods, and antibacterial efficacy, urging comprehensive inquiries to elucidate underlying mechanisms.

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6. Conflict of Interest

The authors declare no competing interest.

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