Antibacterial activity of celery (Apium Graveolens) extract against urinary tract infections.

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Abstract:

Introduction: Urinary tract infection (UTI) is a prevalent microbial infection that affects both males (20%) and females (80%), with an incidence of approximately three occurrences per year. Clinical symptoms include urinary frequency, urgency, suprapubic discomfort, and dysuria. While UTIs can be treated or prevented with antibiotics, continued use may lead to increased resistance and reduced efficacy. For this reason, herbal medicines are considered a viable strategy for treatment and for reducing multidrug resistance. Among these, *Apium graveolens* (celery) is of particular interest. This study aimed to evaluate the antimicrobial activity of *Apium graveolens* L., especially in the context of UTI infections. Materials and Methods: The plants were dried and subjected to hydroalcoholic extraction, followed by evaporation of the ethanol to obtain a dry powder. Phytochemical screening was conducted to determine the contents of tannins, total polyphenols, and total flavonoids. Sample Collection: 100 midstream urine samples were collected and inoculated onto three types of agar media: nutrient agar, MacConkey agar, and blood agar. Media Preparation: Media were prepared according to the manufacturer's instructions. Bacterial Isolation: Isolated bacteria were identified using selective, differential, and general media based on colonial morphology, Gram staining, and biochemical tests. Results: In a study involving 100 UTI patients, 82% were female and 18% were male, with bacterial growth observed in 94 samples. *Escherichia coli* was the predominant pathogen (57.44%), followed by *Klebsiella* spp. (17.02%), *Streptococcus* spp. (10.6%), and other less common bacteria. *Apium graveolens* demonstrated promising dose-dependent antibacterial activity against various bacterial species, achieving inhibitory zones ranging from 5.5 mm to 14 mm, with notable effectiveness. Its impact on *Proteus* spp. was powerful, matching the efficacy of amoxicillin. Conclusion: According to this study, hydroalcoholic extraction of *Apium graveolens* significantly reduces bacterial infections, indicating its potential role in the management of UTIs.

Keywords: UTI, celery, *Apium Graveolens*, antibiotics, amoxicillin

النشاط المضاد للبكتيريا لمستخلص الكرفس ضد التهابات المسالك البولية

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الخلاصة:
المقدمة: يُعدّ التهاب المسالك البولية من الالتهابات الميكروبية الشائعة التي تصيب كلًا من الذكور (20%) والإناث (80%)، مع معدل إصابة يُقدّر بحوالي ثلاث مرات سنويًا. تشمل ا.لأعراض السريرية لهذا المرض تكرار التبول، والإلحاح البولي، والانزعاج فوق العانة، وعسر التبول. وعلى الرغم من إمكانية علاج أو الوقاية من التهابات المسالك البولية باستخدام المضادات الحيوية، إلا أن الاستخدام المطوّل لها قد يؤدي إلى زيادة مقاومة البكتيريا وتقليل فعاليتها. لذلك، تُعدّ الأدوية العشبية بديلاً واعدًا للعلاج والحدّ من مقاومة الأدوية المتعددة. ومن بين هذه الأعشاب، حظي نبات الكرفس باهتمام خاص. يهدفُ هذا البحث إلى تقييم النشاط المضاد للميكروبات لنبات الكرفس، لا سيما في سياق التهابات المسالك البولية. المواد والطرق: تمّ تجفيف الجزء الهوائي من النباتات وإخضاعه لعملية استخلاص كحولي مائي، تلتها عملية تبخير الإيثانول للحصول على مسحوق جاف. كما تمّ إجراء التحليل الكيميائي النباتي لتحديد محتوى العفصات، والبوليفينولات الكلية، والفلافونويدات الكليةز تمّ جمع 100 عينة بولية في منتصف التبول وزُرعت على ثلاثة أنواع من الأوساط الزراعية: أجار المغذيات، وأجار ماكونكي، وأجار الدم. تمّ تحضير الأوساط وفقًا لتعليمات الشركة المصنعة. تمّ تحديد البكتيريا المعزولة باستخدام الأوساط الانتقائية والتفريقية والعامة، بالاعتماد على الخصائص المورفولوجية للمستعمرات، وصبغة جرام، والاختبارات البيوكيميائية. النتائج: من بين 100 مريضًا مصابًا بالتهاب المسالك البولية، كانت نسبة الإناث 82% والذكور 18%، مع ملاحظة نمو بكتيري في 94 عينة. كان الإشريكية القولونية هي المُمْرِض الأكثر انتشارًا (57.44%)، تليها الكلبسيلا بنسبة 17.02%، والمكورات العقدية بنسبة 10.6%، بالإضافة إلى بكتيريا أخرى أقل شيوعًا. أظهر نبات الكرفس نشاطًا مضادًا للبكتيريا يعتمد على الجرعة ضد أنواع بكتيرية متعددة، حيث تراوحت مناطق التثبيط بين 5.5 ملم و14 ملم. كان تأثيره على بروتيوس قويًا بشكل خاص، وقابلًا للمقارنة مع فعالية الأموكسيسيلين. الاستنتاج: تشير هذه الدراسة إلى أن المستخلصات الكحولية المائية لنبات الكرفس تمتلك تأثيرًا مضادًا للبكتيريا بشكل ملحوظ، مما يشير إلى دوره المحتمل في إدارة التهابات المسالك البولية.

الكلمات المفتاحية: التهاب المسالك البولية، الكرفس ، المضادات الحيوية، الأموكسيسيلين

Introduction:

Urinary tract infection (UTI) is a prevalent microbial infection that affects individuals of all ages and genders, leading to inflammation in the urinary tract. The spectrum of these infections varies from mild bladder inflammation, known as cystitis, to more severe instances of urosepsis shock(1). UTIs are prevalent among approximately 20% of the male demographic throughout their lives. UTI within the general population ranges from 0.9 to 2.4 cases per 1000 men under the age of 55, increasing to as much as 7.7 per 1000 in men aged 85 years and older(2). UTIs commonly lead to bacteremia and recurrent infections in this cohort, making them a significant contributor to antibiotic consumption in primary care, being the second most frequent reason for such medication usage (3). UTIs are a common ailment among women, occurring at various life stages. Recurring UTIs in females are characterised by experiencing a minimum of two UTIs within a six-month timeframe or a minimum of three UTIs within a twelve-month period. Studies suggest that approximately 25-50% of all UTIs are recurrent in women, who are more susceptible to UTIs compared to men. It is estimated that between 30% and 50% of women over fifty are affected by UTIs(4, 5). UTIs are commonly categorised as lower or upper, as well as complicated or uncomplicated, depending on the specific location of the infection within the urinary tract and the individual's overall health status (6). Upper UTIs primarily impact the kidneys and ureters, as seen in cases such as pyelonephritis, whereas lower UTIs typically involve the urethra and bladder(7). Urine frequency, urgency, suprapubic discomfort, and dysuria are the most prevalent clinical manifestations of UTIs (8). Bacteria are the primary etiological agents of UTI; however, less commonly, other microorganisms, including fungi and certain viruses, have been documented as causative agents of UTIs. *Uropathogenic Escherichia coli* (UPEC) is the predominant causative pathogen for both uncomplicated (uUTIs) and complicated (cUTIs) urinary tract infections and is considered in 70-95% of the cases, followed by Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis, and various

Staphylococcus species, also identified as significant contributors(9). Conventional diagnostic techniques relying on culturing necessitate specialised expertise, controlled environments for urine specimen culturing, and dedicated analytical settings, rendering them time-intensive. Conversely, optical devices present notable advantages such as heightened sensitivity, ease of use, and portability(10). Antibiotic therapy aims to alleviate symptoms, prevent sepsis, and lower the risk of complications. Early initiation after specimen collection is crucial. Rapid treatment is vital for patients with specific conditions. Local susceptibility patterns should guide empirical antibiotic therapy. Accurate prescription of beta-lactams, aminoglycosides, quinolones, sulfonamides, tetracyclines, penicillins, and cephalosporins groups of antibiotics are recommended for empirical therapy, third-generation cephalosporins are suggested for outpatient care, and aminoglycosides for inpatient treatment(11). Under monitoring, ampicillin is recommended for infants under three months(12). Antibiotic de-escalation is advised once urine culture results are available (12). The growth of antibiotic resistance is exacerbating, likely due to the rise in multidrug-resistant bacterial strains and the indiscriminate administration of broad-spectrum antibiotics and empirical therapies, leading to worsened treatment expenses and hospital admissions. It is imperative to implement antibiotic stewardship in healthcare settings to moderate the occurrence of infection incidents that may precipitate systemic inflammatory response syndrome or sepsis and pose a threat to urological patients (13). The emergence of resistance, undesired effects of antibiotics, and related issues necessitate establishing a research framework to explore alternative strategies for managing UTIs. Natural modalities have been widely employed to treat various ailments to alleviate symptoms and enhance overall well-being; Herbal remedies may prove efficacious upon initial presentation of infection, as well as for short-term prophylaxis. Utilising vitamins, trace elements, and/or carbohydrates represents a viable method for UTI prevention, and when combined with other antibacterial agents, demonstrates favourable outcomes. Probiotics exhibit considerable promise in addressing the risks associated with antibiotic misuse and the proliferation of antibiotic-resistant pathogens. This investigation could prove valuable in formulating an effective approach to UTI treatment(14). Celery, scientifically known as *Apium graveolens L.*, exhibits versatility as an herb, with its agricultural, medicinal, and economic significance extending globally. Belonging to the family Apiaceae, this annual plant is cultivated across Europe and in various regions of Asia and Africa(15). The utilisation of Apium plants' leaves as both a source of nutrition and for medicinal purposes has been documented, showcasing their abundance in bioactive compounds like polyphenols, coumarins, β-ocimene, tocopherol and flavonoids, which are responsible for their therapeutic effects(16). The application of various plant components such as seeds, leaves, stems, roots, and essential oils in traditional medicine is widespread for addressing various health conditions, including hypertension, diabetes, asthma, gastrointestinal infections, bronchitis, and hepatitis. Moreover, the anti-inflammatory properties of celery plants have been noted, demonstrating efficacy in the management of bronchitis and hepatitis(17). This study aimed to evaluate the antimicrobial activity of *Apium graveolens*, especially in UTI infection.

Materials and Methods

Preparations of extracts: the aerial part of the plants dried under shad and then crashed in to fine powder, the crashed plants immersed in hydroalcoholic solvent (Ethanol: water 70:30) for 72hours, afterward, the solvent was filtered using Whatman filter paper No. 1, then used

rotary evaporate for evaporating the ethanol, the resulting extract is subjected to lyophilization until a dry powder was obtained, the dry powder stored in -4C until used.

Phytochemical screening:

Determination of tannins: The crude seed extract (0.5 g) underwent a process of boiling in 20ml of water, followed by cooling and filtration. Subsequently, approximately 1% ferric chloride solution was added, and the sample was monitored for the development of a blue-black or brownish-green hue. The emergence of the specified colour signified the existence of tannins(18).

Determination of total polyphenol content: Folin-Ciocalteu reagent was used to determine total phenolic content in the subject plant fractions with Gallic acid as reference. Crude fractions were mixed with water and FCR, and Na2CO3 solution was added. The mixture was heated for 2 hours, and the absorbance was measured at 765 nm. Total polyphenols were evaluated by comparison with Gallic acid(19).

Determination of total flavonoid content: The study assessed flavonoid content in a plant using the colourimetric method with quercetin as a standard. Plant samples were mixed with NaNO2, AlCl3, and NaOH, then warmed and absorbed by a spectrophotometer. Flavones were quantified as quercetin equivalents in mg/g of extract(20).

Sample Collection: At Smart Tower Hospital, 100 midstream urine samples were collected from male and female patients with urinary tract infections. The samples were inoculated onto three types of agar media: nutrient agar, MacConkey agar, and blood agar. After inoculation, the agar plates were placed in an incubator at 37°C.

Media preparation: Media preparations **were prepared according to the manufacturer's instructions, which involved mixing various agar types with distilled water, boiling, and sterilising** them for bacterial growth. Blood agar requires adding blood before being poured into Petri dishes. MacConkey agar is prepared similarly but without blood. Nutrient agar and Mannitol salt agar follow similar procedures with different ingredients. Eosin-methylene blue (EMB) is used for E. coli confirmation, while Nutrient broth is prepared for antimicrobial testing. Mueller-Hinton Agar is used for testing celery's antimicrobial activity against UTI-causing bacteria.

Bacterial identification: Eight clinical isolates of uropathogenic bacteria were obtained as a result of biochemical tests and gram stains, including gram-negative bacteria (E. coli, Klebsiella pneumoniae, Enterobacter spp., Proteus spp., and Serratia spp.) and gram-positive bacteria (Streptococcus spp., Staphylococcus aureus, and *Staphylococcus epidermidis*).

Study design: The urine samples were collected from the admitted patients of different genders who suffered from UTIs, and then different bacterial species were isolated according to agar media, after the isolation. Each Bacterial species was colonized on five different media: three different extract dilutions (AG0.5: Celery extract at 0.05g, AG1: Celery extract at 0.1g, AG2: Celery extract at 0.2g, and AG4: Celery extract at 0.4g), along with amoxicillin as a positive control (AMOX, 0.5g) and DMSO as a negative control.

Determination of Minimum Bactericidal Concentration: To determine the minimum bactericidal concentration (MBC) values, a volume of MIC mixtures without any visible growth was placed onto Mueller-Hinton plates and allowed to grow at 37°C for 24 hours(21). Following incubation, bacterial growth was quantified. MBC was defined as the lowest concentration of the test compound where no bacterial colonies were seen, and it represented absolute killi ng of bacteria and not inhibition

Antibacterial study: The antibacterial efficacy of the plant extract was evaluated

using the Kirby-Bauer disc diffusion method, following the guidelines established by the National Committee for Clinical Laboratory Standards. Mueller-Hinton agar (MHA) was sterilised, poured into Petri dishes, and utilised for the assay. Bacterial cultures were prepared from overnight growth and standardised to a turbidity equivalent to 0.5 McFarland. The surface of the MHA plates was inoculated with the bacterial suspension using a sterile cotton swab to ensure uniform distribution. Sterile discs (approximately 6 mm in diameter) were prepared from Whatman No. 1 filter paper, subsequently sterilised and desiccated. Crude plant extracts were then serially diluted to final concentrations of 50, 200, and 400 mg/mL before being impregnated onto the discs, which were subsequently air-dried. Negative control discs containing dimethyl sulfoxide (DMSO) and positive control discs containing amoxicillin were prepared similarly. The impregnated discs were then carefully placed onto the inoculated agar plates and allowed to pre-diffuse at room temperature for 15 minutes before incubation at 37°C.

Data analysis: Data obtained from the experiment were analysed using Prism, version 9, ANOVA followed by Tukey's Multiple Comparison tests.

Results:

3.1. Herbal identification:

Totally, 12 g of powder was obtained from 1000 g of crude herbal, and after screening, the availability of tannins, total polyphenol content, and total flavonoid content was confirmed. The total phenolic content is 57mg gallic/gram, the Total flavonoids content is 24mg rutin /gm, and the catechin content is 0.365 g/gm.

3.2. Distribution of UTI patients in relation to their sex: 100 urine samples were collected from patients suffering from UTI; among them, 82% were female and 18% were male. In six samples, no bacterial growth was observed on the agar, but in the remaining samples, bacteria grew on the agar, as shown in Table 1

Table 1: Distribution of UTI patients in relation to their sex:

Gender	No. of growth cases	Percent
Male	16	18%
Female	78	82%

3.3 Distribution of bacterial isolates from urine sample: A total of eight clinical isolates of uropathogenic bacteria were obtained from biochemical tests and Gram stains. Out of these isolates, 80.82% are gram-negative bacteria, which include (E. coli, Klebsiella pneumoniae, Enterobacter spp, Proteus spp., and Serratia spp.), and 19.6% are gram-positive bacteria, which include (*Streptococcus spp., Staphylococcus aureus, Staphylococcus epidermidis*). Also, the results indicated that *Escherichia coli* was the predominant isolate, accounting for 54 (57.44%) of the positive urine samples. This was followed by *Klebsiella* spp. with 16 (17.02%), *Streptococcus* spp. with 10 (10.64%), and *Staphylococcus aureus* and *Staphylococcus epidermidis*, each contributing 4 (4.26%). Additionally, *Enterobacter* spp., *Proteus* spp., and *Serratia* spp. were each identified in 2 (2.12%) of the samples. Table 2.

Table 2: The percentage of bacteria isolated from the urine of the UTI patients

No.	Bacteria isolate	No. of isolate	Percent
1	*E. coli*	54	57.44%
2	*Klebsiella spp*	16	17.02%
3	*Streptococcus spp*	10	10.64%
4	*Staphylococcus aureus*	4	4.26%
5	*Staphylococcus epidermidis*	4	4.26%
6	*Enterobacter spp*	2	2.12%
7	*Proteus spp*	2	2.12%
8	*Serratia*	2	2.12%
	Total	94	100%

3.4 Isolation and identification:

Escherichia coli, a Gram-negative bacterium, forms pink to dark pink, dry colonies with a doughnut-shaped morphology on MacConkey agar. These colonies are surrounded by a dark pink zone due to the precipitation of bile salts. Confirmation of E. coli can be performed by culturing on eosin methylene blue (EMB) agar and conducting biochemical tests, including urease negativity, catalase positivity, oxidase negativity, and citrate negativity (see Figure 1A). Klebsiella pneumoniae, another Gram-negative bacterium, produces large, dome-shaped, mucoid colonies on MacConkey agar, appearing pink due to lactose fermentation. Confirmation of K. pneumoniae involves biochemical tests, including urease positivity, a Kligler's Iron Agar (KIA) test showing an acid/acid (A/A) reaction, a positive catalase test, a negative oxidase test, a positive Simmons citrate test, and negative methyl red and indole tests (see Figure 1B). Staphylococcus aureus forms golden-yellow colonies on blood agar. Its presence is confirmed using Mannitol Salt Agar (MSA), where it produces yellow colonies surrounded by yellow zones due to mannitol fermentation. Serratia species form dark red colonies on MacConkey agar, contrasting with the light pink background of the medium. They also grow on nutrient agar, where they exhibit characteristic orange pigmentation. Proteus species grow well on both blood agar and MacConkey agar, demonstrating a distinctive swarming growth pattern on blood agar due to their high motility. The antimicrobial efficiency of the test co mpound against E. coli, Klebsiella, Staphylococcus aureus, Enterobacter, Proteus, and Streptococcus was established using the disc diffusion technique in the research. Higher concentrations (400 mg) provided larger inhibition zones, indicating a concentration-related effect. Gram-positive bacteria (S. aureus, Streptococcus) were more sensitive, while Gram-negative strains responded variably. The positive control demonstrated antimicrobial activity, and the negative control lacked inhibition. Overall, the test agent showed considerable antibacterial ac tivity, especially at higher concentrations, and thus deserves further investigation through MIC and time-kill tests. (Figure 2)

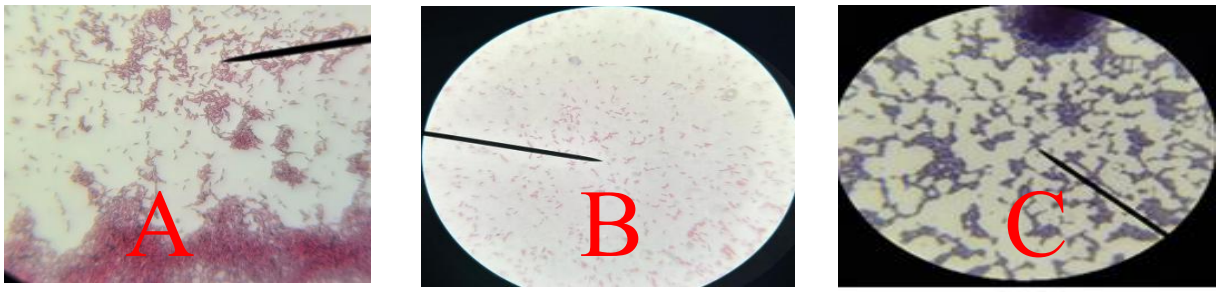

Figure 1: Gram stain of bacteria isolates
A: Escherichia coli, B: Klebsiella pneumoniae, C: *Gram stain of bacteria isolates.*

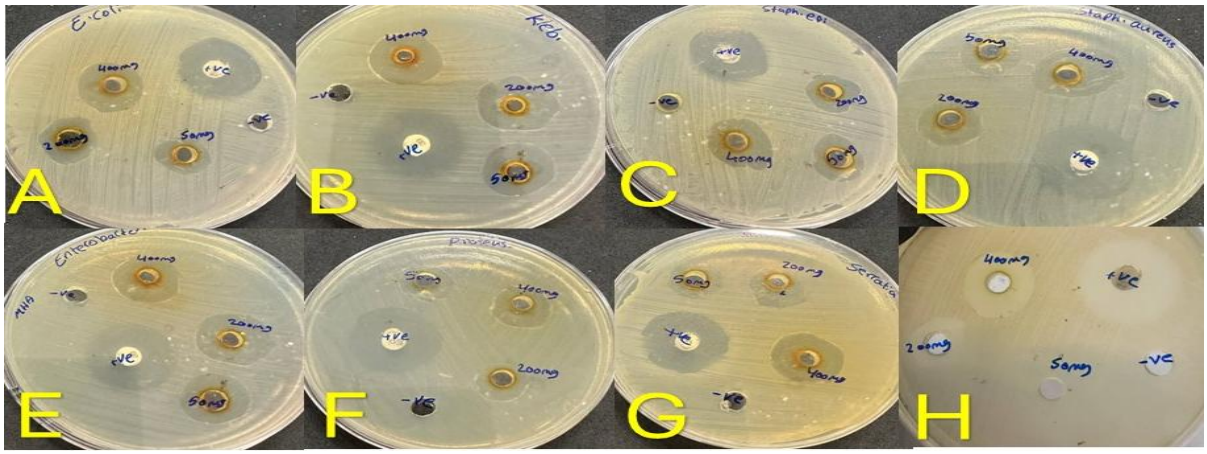

Figure 2: Antibacterial activity of *Apium graveolens* extract on different species of isolates with inhibitory zone
Note: A: *E. coli,* B: *Klebsiella pneumoniae,* C: *Staphylococcus epidermidis.* D: *Staphylococcus aureus,* E: *Enterobacter spp,* F: *Proteus spp.,* G: *Serratia spp.*, H: *Staphylococcus epidermidis*

Negative control DMSO does not have or has very poor inhibition, confirming that neither solvent is involved in antibacterial activity. Positive control Amoxicillin always shows the maximum zone of inhibition among all the bacteria, indicating excellent antibacterial activity.

Apium graveolens extract in different concentrations (50, 200, and 400 mg/ml) exhibits varying levels of antibacterial activity. Higher doses (AG4) were found to form larger inhibition zones than lower doses (AG0.5), indicating dose-dependent antibacterial activity. Against some bacteria (e.g., Proteus sp., Enterobacter aerogenes), *Apium graveolens* extract and amoxicillin yield no statistically significant difference, which indicates moderate activity. While against some other bacteria (e.g., E. coli, Klebsiella pneumoniae, Staphylococcus epidermidis), *Apium graveolens* extract in higher doses had significant antibacterial activity, yet less than with amoxicillin.

Effect of *Apium graveolens on E. coli bacteria*: The antibacterial activity of different doses of the herbal extract was significantly observed compared to the DMSO control groups. The inhibitory zone ranged from 8 to 12mm; however, it did not reach the level of the activity of Amoxicillin, as shown in Figure 3.

Effect of *Apium graveolens on Klebsiella pneumoniae*: Regarding the antibacterial activity of this herbal extract on the *Klebsiella pneumoniae*, the inhibitory zone was approximately 8mm at the lower dose and increased to 12 mm at the higher dose. However, compared to Amoxicillin, which reached an inhibitory zone of 18 mm.

Effect of *Apium graveolens on Staphylococcus aureus:* Regarding the antibacterial activity of *Apium graveolens*

against *Staphylococcus aureus*, the efficacy of higher doses was significant. The inhibitory zone at lower doses was approximately 7 mm; at higher doses, it reached 14 mm. However, Amoxicillin demonstrated greater activity, with an inhibitory zone of about 20 mm.

Effect of *Apium graveolens* on *Staphylococcus epidermidis*: The antibacterial activity of *Apium graveolens on Staphylococcus epidermidis* gradually increased from a lower dose (6mm) to a high dose (about 12mm), but it was not as effective as amoxicillin (18mm).

Effect of *Apium graveolens* on Proteus SPP: The efficacy of *Apium graveolens* extract on this species is greater than that of the other species, as can be seen in the inhibitory zone, which in lower doses is about 8mm and was elevated in high doses to 12mm. This is similar to the effect of amoxicillin on the Proteus SPP. In other words, there is no significant difference in the inhibitory zone of amoxicillin and *Apium graveolens.*

Effect of *Apium graveolens* on Serratia spp: The inhibitory zone of *Apium graveolens* extract on Serratia spp was about 5.5mm at lower doses. In comparison, it was elevated to 13mm at high doses. However, Amoxicillin exhibited a significantly larger inhibitory zone of about 21 mm.

Effect of *Apium graveolens* on *Enterobacter spp*: The inhibitory zone of *Apium graveolens* extract on *Enterobacter spp*. was about 9mm at lower doses and did not increase significantly with increased dose, except for the high dose, where it reached approximately 13 mm. The amoxicillin indicated a greater effect with an inhibitory zone of about 21mm.

Effect of *Apium graveolens* on *Streptococcus spp*: The inhibitory zone of *Apium graveolens* on *Streptococcus spp* is about 9mm in lower doses, and it dramatically increased with increased dose, as can be seen in high doses, where it reached 14mm, while the inhibitory zone of amoxicillin is about 20mm.

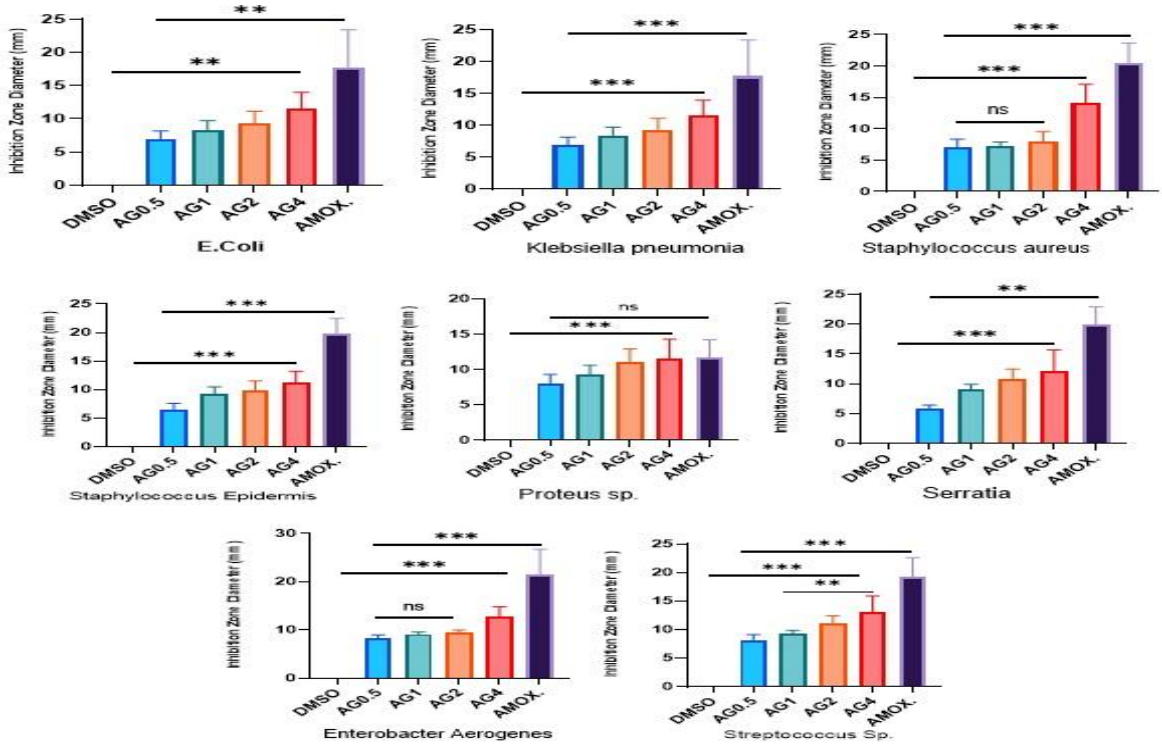

Figure 3: The antibacterial activity of Apium graveolens on different species of bacteria
Note: DMSO: Dimethyl sulfoxide, AG: *Apium graveolens, AMOX: Amoxicillin,*
: P < 0.05, **:P < 0.01, ***: P < 0.001

Discussion:

Based on the available data and literature, antimicrobial resistance is perceived as a significant global challenge. In the search for novel therapeutic agents and methodologies to address this issue, phytochemical compounds are being explored for their potential contributions. Urinary tract infections (UTIs) are one of the prevalent infection types, impacting around 20% of individuals.(3).

To the best of the author's understanding, *Apium graveolens*, due to its diverse array of chemical constituents, may exhibit antibacterial properties, prompting the execution of this investigation. This study also reveals that the incidence of urinary tract infections (UTIs) is higher among females compared to males, a conclusion that is consistent with the findings of Gebretensaie, Yosef, et al. (2023)(22).

In the present study, eight different strains of bacteria were identified using various biochemical tests and Gram staining techniques. Among these strains, approximately 80.7% of the samples were identified as *gram-negative* bacteria, while the remaining 19.3% were identified as *gram-positi*ve bacteria. Notably, *Escherichia coli* emerged as the most prevalent uropathogenic bacterium, constituting 57.44% of the isolated strains. This finding aligns with the research outcomes Niranjan, V., and A. Malini reported in 2014.(23), Following *E. coli, Klebsiella pneumoniae* was identified as the next most common strain at a rate of 17.02%, a result that corresponds with the observations made by Mansour and Amin in 2009(24). *Subsequently, Streptococcus species* were identified with a prevalence of 10.63%, whereas *Staphylococcus aureus* and *Staphylococcus epidermidis* were observed at a frequency of 4.26%. Moreover, *Enterobacter, Proteus*, and *Serratia* species were individually detected at a frequency of 2.12%.

In terms of the study on antibacterial properties, it was observed that all bacterial strains, including *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterobacter aeruginosa*, *Streptococcus* spp., *Serratia* spp., *Proteus* spp., and *Klebsiella pneumoniae*, exhibited sensitivity to amoxicillin, which was utilised as the positive control. The inhibition zones ranged from 11 to 24 mm. This finding is consistent with the results reported by Beytur et al. (2015) (25). And those obtained by Aljeboury et al. (2017) (26). *Staphylococcus aureus* and *Staphylococcus epidermidis* demonstrated notable sensitivity towards amoxicillin, with inhibition zones ranging from 24 to 20mm, exceeding the sensitivity observed in the other bacterial strains examined. Conversely, all isolates exhibited considerable resistance to Dimethyl sulfoxide (DMSO), employed as the negative control, resulting in unfavourable outcomes consistently across all tested bacterial strains.

The findings of this investigation indicate that the efficacy of *Apium graveolens* extract in treating urinary tract infection (UTI)-associated bacteria can be assessed by identifying specific bacterial species. The study suggests that *Apium graveolens* exerts a significant antibacterial effect on various isolated bacteria, with an increase in dosage leading to a proportional expansion of the inhibitory zone. This trend is particularly evident in *Staphylococcus aureus, Enterobacter aeruginosa, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumoniae, Streptococcus spp., Proteus spp., and Serratia spp*. These results are consistent with the findings of Sarshar et al. (2018)(27), and Alshwaikh et al. (2014)(28), and Mohammed Ghazi (2016) (29). Despite its significant antibacterial activity compared to dimethyl sulfoxide (DMSO), *Apium graveolens* demonstrates particularly strong effects against *Proteus spp*., resembling the efficacy of amoxicillin. However, its impact on other bacterial species does not reach the

inhibitory levels observed with amoxicillin. Notably, increasing the dosage significantly enhances the inhibition of *E. coli, Klebsiella pneumoniae, Staphylococcus aureus, and Serratia spp.*. In contrast, no significant changes are observed in *Proteus spp.* and *Enterobacter aeruginosa* with higher doses. The exact mechanism of action of *Apium graveolens* remains unclear; however, it may be linked to various bioactive compounds present in the herb, including flavonoids, polyphenols, and tannins. This study identified these components, corroborating the findings of Marouf et al. (2022)(30). Studies have shown that flavonoids can impair bacterial defence mechanisms by inhibiting efflux pumps, disrupting bacterial cell membranes, and interfering with key enzymes such as β-lactamases and topoisomerases responsible for antibiotic resistance. This suggests that flavonoids may serve as potential agents in preventing the emergence of antibiotic-resistant bacteria, as supported by research from Daglia et al. (2012) (31). Additionally, *Apium graveolens* contains polyphenols, which are believed to exert antibacterial effects through three primary mechanisms: direct bacterial killing, enhancement of antibiotic efficacy and bacterial virulence reduction. These findings align with Xie et al.'s (2017) (32) (32), tannins, another significant component of *Apium graveolens*, also exhibit antibacterial activity. The antibacterial effects of tannins have been supported by Noce et al. (2021)(33). While the exact mechanism remains unclear, tannins are thought to inhibit bacterial cell wall synthesis and cause structural damage (34). The findings of this study further substantiate the antibacterial activity of *Apium graveolens*. Its broad-spectrum efficacy is attributed to the plant's diverse array of bioactive constituents, demonstrating effectiveness even against the varied bacterial populations isolated from the urine samples of UTI patients.

Conclusion:

Anti-bacterial resistance is considered a health disaster and continue looking for reducing this problem is crucial, herbal medicine has been one of the most essential martial in the past and now for reducing this issue, because of their low side effect and acceptance by users and their phytochemical compounds, in this context, *Apium graveolens* has emerged as a notable antibacterial agent. This study demonstrates that hydroalcoholic extracts of *Apium graveolens* significantly reduce bacterial infections in patients with urinary tract infections (UTIs). However, further investigation, particularly in vivo studies, is needed to determine optimal dosages, mechanisms of action, and additional therapeutic roles of this herbal extract.

Conflict of interest:

There is no conflict of interest.

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