



**ANTIOXIDANT, PHYTOCHEMICAL, ANTIBACTERIAL EFFECT OF SCOPARIA
DULCIS ON UROPATHOGENS ISOLATED FROM CLINICAL SAMPLES**

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ABSTRACT

Introduction: Urinary tract infection has evolved into an infection that is influenced by the treatment process, specifically, the use of antibiotics, resulting in the emergence of multi-drug resistant strains derived from the normal flora. Furthermore, introducing gastrointestinal pathogens has exacerbated the situation, compounding the challenges faced in treating and preventing such infections. Consequently, it is crucial to accurately characterize these uropathogens to address the issue of infections effectively.

1. INTRODUCTION

It is worth noting that urinary tract infection is widely recognized as the most prevalent bacterial infection, as highlighted by Betsy Foxman (2002). The study of urinary tract infections has made significant progress in recent centuries; however, its exploration can be traced back as early as 3000 BC. During that era, the origin of this infection was not attributed to bacteria, and instead, various myths were believed. The treatment of urinary tract infections was administered by Greek priests, who perceived it as a divine directive for healing. The study of the urinary tract in past decades aimed to identify the presence of different bacteria and other microbes responsible for these infections. Presently, research has advanced to the stage where multi-drug resistant strains of uropathogenic bacteria are prevalent.

It is stated that every woman will experience a urinary tract infection at some point in her life Betsy Foxman (2002). It is concluded that the probability of a woman encountering a urinary tract infection is 3:1, meaning that out of three women, at least one will experience such an infection. This finding emphasizes the significant socio-personal impact of urinary tract infections. The continuous use of antimicrobial agents to combat infections can contribute to the development of multi-drug resistance in common uropathogenic bacteria. Thus, it is imperative to identify effective remedies for both normal and multi-drug-resistant strains that cause urinary tract infections.

Urinary tract infections (UTI) occur when uropathogens, which reside in the gut, establish colonies in the urethra

and subsequently invade the bladder through the utilization of specific adhesins. If the host's inflammatory response fails to eliminate all bacteria, these pathogens can proliferate, producing toxins and enzymes that enhance their persistence. Further colonization of the kidneys can lead to bacteremia if the pathogen breaches the protective epithelial barrier of the kidneys. In terms of classification, urinary tract infections can be categorized as either upper UTIs or lower UTIs. Upper UTIs refer to infections that affect the upper parts of the bladder, while lower UTIs are characterized by infections in the gallbladder. Upper UTIs are considered to be complicated UTIs, as they can extend to the kidneys and potentially result in kidney failure. The presence or absence of symptoms in UTIs depends on the infectious organism and its virulence factor. Notably, the bacteria responsible for UTIs often possess multiple virulence factors, which contribute to their pathogenicity, as highlighted by Roger D. Kleina et al., (2020).

To summarize, the treatment-mediated nature of urinary tract infections, coupled with the emergence of multi-drug resistant strains from the normal flora, has underscored the importance of characterizing uropathogens. The prevalence of urinary tract infections, particularly among women, has also highlighted the need for effective remedies to address this socio-personal problem. Understanding the mechanisms of infection and the virulence factors associated with uropathogenic bacteria is crucial in developing targeted interventions. By comprehending the intricacies of urinary tract infections, it can strive towards more effective treatment and prevention strategies, ultimately mitigating the

impact of these infections on individuals and society as a whole.

Urinary tract infections (UTIs) have the potential to manifest either asymptotically or with symptoms, contingent upon the existence or absence of particular indications. In the case of young, robust females, the presence of symptoms can serve as an aid in the diagnostic process. Nevertheless, UTIs are less prevalent in the pediatric population. Cystitis, commonly referred to as a lower urinary tract infection (UTI) or bladder infection, specifically targets the bladder and manifests with various symptoms. These symptoms include pressure in the lower pelvis, dysuria, polyuria (frequent urination), urinary urgency, nocturia (urination during the night), and haematuria. Cystitis can be further categorized based on its etiology and therapeutic approach. Among females, traumatic cystitis is the most common form, which affects the bladder. Subsequently, bacterial cystitis follows, where in coliform bacteria are transmitted to the bladder from the bowel through the urethra (Ranganathan Vasudevan, 2014).

During acute cystitis, the prevalent pathogen Uropathogenic *Escherichia coli* (UPEC) divides within the cytoplasm of bladder epithelial cells. However, oral antibiotics are not entirely effective against UPEC due to the presence of pili, which facilitate the microbe's growth and enable it to cause high infection levels by multiplying on the same site or at multiple sites. The normal flora present in the urinary tract serves as the primary reason for the occurrence of UTIs. The normal or uncomplicated bacterial UTIs encompass both gram-positive and gram-negative bacteria. The gram-negative group includes *E. coli*, *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, group B *Streptococcus* (GBS), *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. *E. coli* is the most common cause of UTIs and is responsible for both uncomplicated and complicated cases. *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *E. coli* are among the major causative agents of complicated UTIs. Complicated UTIs can also infect the genital tract. Studies about UTIs have concluded that they primarily occur in individuals above the age of 60, although they occasionally affect teenagers and young adults as well. Pregnant women are particularly susceptible to UTIs, which may lead to severe complications such as pyelonephritis, premature delivery, and fetal mortality (Betsy Foxman *et al.*, 2002).

Multidrug resistance (MDR) refers to the ability of certain microorganisms to withstand the effects of multiple antimicrobial agents. MDR encompasses resistance to various antibacterial, antifungal, antiviral, and anti-parasitic drugs. The prevalence of MDR bacterial isolates among UTI patients, along with the antibiotic resistance pattern and the transfer of multidrug resistance phenotype in *E. coli* through conjugation, has been extensively studied. The first indications of

antibiotic resistance surfaced shortly after the discovery of penicillin. In 1940, Abraham and Chain reported that an *E. coli* strain possessed the ability to render penicillin ineffective by producing penicillinase.

Recent data has unveiled a mounting number of isolates with multidrug resistance (MDR) responsible for outpatient urinary tract infections (UTIs), consequently presenting a greater challenge in treatment and eradication. Risk factors associated with UTIs caused by multidrug-resistant uropathogens encompass prior utilization of antimicrobial agents, exposure to healthcare settings, complicating genitourinary factors, advanced age, recurrent UTIs, and male gender. The mechanism behind bacterial multidrug resistance involves the accumulation of resistance genes, each encoding resistance to a specific agent, on resistance plasmids or transposons. Alternatively, it can occur through the operation of multidrug efflux pumps, each possessing the ability to expel multiple types of drugs, as highlighted in the study conducted by Tomihiko Yasufuku *et al.*, (2011).

The prevalence of biofilm-forming strains of uropathogenic *Escherichia coli* (UPEC), which demonstrate a strong correlation with the Multidrug-Resistant (MDR) phenotype, is noteworthy. As a recommendation, it is of utmost importance to regularly monitor antimicrobial resistance and biofilm formation to determine the most efficacious antibiotics for managing biofilm-associated urinary tract infections (UTIs). UPEC isolates that are capable of forming moderate to strong biofilms exhibited a higher incidence of three adhesion factors (AFGs), thereby suggesting that these factors may contribute to the development of biofilms, as indicated by Hojjatolah Zamani *et al.*, (2017).

Among the 106 isolates examined in the study by Anuja Dahal *et al.* (2023), the most commonly identified uropathogens were *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterococcus spp.* It was found that 74.5% of these isolates were able to produce biofilms, and 70.8% displayed multidrug resistance (MDR). The formation of biofilms grants bacteria numerous advantages, including the acquisition of antibiotic tolerance, the expression of various virulence factors, and heightened resistance against phagocytosis and other host defense mechanisms. In the context of these clinical processes, biofilm formation is the primary culprit behind the persistent nature of infections, even in the presence of appropriate antibiotic therapy and hydrodynamic forces by Andrea Hanna *et al.*, (2003).

The initial step in biofilm formation involves the attachment of bacteria to a surface. The adherence of UPEC strains can be influenced by a wide array of intrinsic factors, such as adhesive proteins, fibers, and exopolysaccharide molecules. The presence and expression of these factors differ from one strain to another. The age and composition of a biofilm

significantly impact the susceptibility of the microorganisms residing within it. As a biofilm matures, it accumulates an extracellular polymeric substance (EPS) matrix, which plays a critical role in its structure and function. This matrix comprises polysaccharides, proteins, and DNA, thereby providing structural stability and protection to the biofilm community, as elucidated by *Maria Kostakiotiet al., (2013)*.

Patients with catheters or vaginal/cervical implants are included in the list of individuals at risk due to the presence of biofilm-forming bacteria on the inner lining of epithelial cells or the surface of the implants. These patients also exhibit the presence of multi-drug resistant strains that cause UTIs. Various factors contribute to the development of UTIs, including holding urine for extended periods, which can lead to the embedding of harmful bacteria in the bladder. Additionally, the presence of kidney stones can cause UTIs by obstructing urine flow, while individuals with diabetes mellitus have high sugar content in their blood, creating favorable conditions for bacterial growth by providing nutrients. Other risk factors for recurring UTIs in women include behavioral factors, susceptibility factors, genetic factors, age-specific factors, pregnancy-related factors, and urinary catheterization, as described by *Rajanbir Kaur et al., (2021)*.

Indian traditional medicines have a significantly high success rate, as evidenced by the utilization of herbs and herbal extracts. One such herb commonly found in the southern region of India is *Scoparia dulcis*, which holds immense medicinal importance. Referred to as the sweet broom herb, *Scoparia dulcis* thrives in tropical and subtropical regions such as India, America, Brazil, the West Indies, and Myanmar. Its habitat primarily encompasses grazed grasslands, wet wastelands, and cultivated lands. Notably, the leaves of *Scoparia dulcis* grow in whorls of three, with varying leaf blade characteristics, particularly on the lower surface. In terms of its root structure, *Scoparia dulcis* possesses taproots that are straight, pale yellow, and accompanied by numerous lateral roots. These roots are remarkably small and assume an obconical shape. Additionally, the seed of *Scoparia dulcis* exhibits a light-demanding nature, requiring appropriate temperatures ranging from 20°C to 30°C for optimal growth (*Ahana Sarkar et al., 2020*).

Within the genus *Scoparia*, there are a total of 10 species, with *Scoparia dulcis* being the sole species found in China (*Yinbong Yang et al., 2023*). Synonyms for *Scoparia dulcis* include *Scoparia grandiflora*, *Scoparia ternate*, *Capraria dulcis*, and *Gratiola micrantha*. The plant yields several compounds, such as Scoparic acid A, Scoparic acid B, Scopadulcic acid A & B, Scopadulciol, and Scopadulin. These compounds possess valuable properties for the treatment of metabolic syndromes, including anti-diabetic, anti-inflammatory, anti-oxidative, and anti-arthritic effects. Furthermore, *Scoparia dulcis* contains a diverse array of components,

such as flavonoids, steroids, and polysaccharides, which contribute to its medicinal properties (*Heng Dao Lin et al., 2023*).

Historically, *Scoparia dulcis* has been employed for alleviating stomach troubles (*Sathyannarayanan 1969*). It is particularly utilized to mitigate discomforts associated with menstruation, menopause, abdominal pain, and gastric diseases, although the precise mechanism of action remains unclear. Despite the advent of modern medicine, many individuals still rely on the medicinal extracts derived from *Scoparia dulcis*, highlighting its enduring significance in traditional healing practices (*MD. Moniruzzaman 2015*). Notably, the entire plant is utilized in the form of a decoction to dissolve stones, often in conjunction with various adjuvants (*Hima Sasidharan et al., 2018*).

As one of the traditional antidiabetic herbs, *Scoparia dulcis* exhibits both antioxidant and antimicrobial activities (*Mishra et al. 2013*). The Brazilians have been utilizing this plant for the treatment of wounds since ancient times (*Hoehne, 1939*). The white flowers of *Scoparia dulcis* possess analgesic and anti-inflammatory properties. Numerous research studies have also highlighted its efficacy in the treatment of diabetes mellitus, making it a highly valuable therapeutic agent. Furthermore, *Scoparia dulcis* has been explored for its potential in treating kidney stones, among other purposes, due to its remarkable therapeutic value (*Sonia et al.*).

Vernacular names

English: Sweet Broom Weed Hindi: Ghoda tulsi Bengali: Bon Dhonia Malayalam: Kallururukki Tamil: Sarakottini Kannada: Mruganh Gida Marathi: Dulas.

Distribution and Botanical features

The favorable growth and blooming of the *Scoparia dulcis* can be observed in tropical and subtropical regions across the globe. These regions encompass various types of landscapes, including grazed grasslands, wet wastelands, and cultivated lands, where the plant thrives abundantly. The leaves of this plant exhibit distinct characteristics, such as being serrated with a tapering base, acute apex, and lanceolated shape. Furthermore, they are arranged in a unique whorled pattern, which adds to the plant's aesthetic appeal.

In terms of texture, the leaves are smooth and hairless on both sides, known as glabrous. Additionally, the leaves emit a pleasant leafy aroma and possess a light green coloration.

The roots and leaves of the *Scoparia dulcis* plant hold significant medicinal value due to the presence of various active compounds. One such compound is the ethanolic extract, which has been found to exhibit anti-inflammatory and analgesic properties. This has led to its utilization by indigenous tribes in Nicaragua, who

employ hot water infusion or decoction of the leaves or the entire plant for treating insect bites, purifying the blood, alleviating fever, and addressing heart problems. The plant is also considered a general tonic, highlighting its diverse range of applications in traditional medicine (The Healing Power of Rainforest Herbs 2005).

The emergence of multi-drug resistance has become a pressing issue on a global scale. To combat the formation of multi-drug resistant (MDR) strains, it is crucial to implement effective strategies for controlling the use of antibiotics. Our research endeavors shed light on the significance of this matter, emphasizing the need for a comprehensive solution. In our study, we specifically focus on exploring the potential of natural therapeutic plant extracts as an alternative treatment for urinary tract infections (UTIs). By investigating the extract derived from the leaves of the *Scoparia dulcis* plant, this study aims to elucidate its lytic action against common UTI-causing bacteria. This investigation delves into the implications and potential benefits of utilizing *Scoparia dulcis* plant extracts in combating uropathogenic bacterial species.

2. Review of literature

Scoparia dulcis is a widely recognized traditional medicinal plant that is utilized in various indigenous systems of medicine. Its distribution extends throughout India. The current investigation presents the phytochemical and antimicrobial characteristics of the methanolic and aqueous leaf extracts of *Scoparia dulcis* against clinically significant human pathogens, namely *Staphylococcus aureus*, *Proteus mirabilis*, *Salmonella typhi*, *Vibrio cholerae*, and *Bacillus subtilis*. (Sophy Jose *et al* 2017). The conducted phytochemical analysis unveiled the presence of flavonoids, glycosides, alkaloids, tannins, steroids, and numerous other metabolites, while saponins were found to be absent. The extract was subjected to a Minimum Inhibitory Concentration (MIC) assay. The methanolic and aqueous extracts exhibited toxicity against all the bacteria, with *V. Cholerae* and *P.mirabilis* being highly susceptible, displaying a zone of inhibition of 4 mm at 10mg/ml and 2 mm at 10mg/ml, respectively, in the agar diffusion method. The broth dilution method demonstrated a more pronounced antimicrobial activity, resulting in 100% inhibition of all the pathogens within the concentration range of 1- 32mg/mL. The MIC for *S. typhi* and *B.subtilis* in the methanolic solution was 32mg/mL, while for *P. mirabilis* it was 16mg/mL, for *S.aureus* it was 8mg/mL, and for *V. cholerae* it was 1mg/mL. In the aqueous solution, the MIC for *S. aureus* and *V. cholerae* was 16mg/mL, for *P.mirabilis* it was 4mg/mL, for *S.typhi* it was 8mg/mL, and for *B.subtilis* it was 32mg/mL.

The traditional knowledge of medicinal plants used for treating urinary tract infections in the Kanyakumari district of Tamil Nadu, India. The data was gathered through in-depth interviews with local inhabitants, elderly individuals with extensive experience, traditional

healers, and vendors of herbal remedies in the Kanyakumari district. A comprehensive total of 29 medicinal plant species from 21 different plant families were identified as being utilized as therapeutic remedies for urinary tract infections by the residents of Kanyakumari district. The medicinal plants were systematically categorized in alphabetical order based on their botanical names, accompanied by additional information such as local names in Tamil, family classification, specific plant parts used for treatment, methods of preparation, and routes of administration (Divya PV *et al.*, 2023). The indigenous knowledge acquired from this survey has the potential to assist the scientific community in exploring these plants further to uncover bioactive compounds that could potentially serve as effective treatments for urinary tract infections in the future.

In their comprehensive investigation, Sirak Biset *et al.* (2020) conducted a thorough examination of the prevalence of Multidrug Resistance (MDR) among urinary pathogens in the context of Urinary Tract Infections (UTIs) during pregnancy. They emphasized the importance of considering antibacterial susceptibility testing results when determining the appropriate treatment for UTIs in pregnant individuals. Furthermore, the researchers highlighted the significance of regular monitoring to effectively identify and manage drug-resistant strains, particularly those that are classified as extended-spectrum beta-lactamase strains. The authors also underscored the need for continuous monitoring of antibiotic consumption in pregnant women. Notably, the study findings revealed that certain antibiotics, such as Nitrofurantoin, gentamicin, amikacin, and Ciprofloxacin/Norfloxacin, demonstrate heightened activity against uropathogenic bacterial pathogens. These findings shed light on potential treatment options for UTIs during pregnancy and emphasize the importance of tailored and vigilant management strategies to mitigate the risks associated with MDR. Undoubtedly, this research contributes to the growing body of knowledge in this field and offers valuable insights for healthcare professionals involved in the care of pregnant individuals experiencing UTIs. Overall, this study serves as a crucial stepping stone toward understanding and addressing the challenges posed by MDR in the context of UTIs during pregnancy.

The treatment involved the use of herbal remedies without a clear understanding of the underlying mechanisms. The belief at that time was that urinary calculi were caused by an imbalance of the four body fluids. Based on this theory, urinary disorders were diagnosed and treated using conservative methods that were advocated by Greek physicians. However, over time, surgical techniques such as surgical lithotripsy and catheterization were improved to treat stones and urinary retention, respectively. Additionally, advancements were made in the field of urological endoscopy, which allowed for a more detailed classification of urological diseases.

The results obtained from urological endoscopy were used to interpret and classify urinary tract infections (*J Curtis Nickel et al., 2005*). Interestingly, the study provided a detailed description of urinary tract infections, even though the causative microorganism was unknown.

Urinary tract infections (UTIs), pose a significant public health challenge and are caused by various pathogens. Among these pathogens, *Escherichia coli*, *S. pneumoniae*, *S. peculiaris*, *Enterococcus faecalis*, and *Staphylococcus faecalis* were found to be the most common causative agents. Unfortunately, the high recurrence rates of these urological pathogens, coupled with the increasing antimicrobial resistance, have resulted in a growing economic burden associated with UTIs (*Ana L. Flores 2015*). To understand the complex molecular interactions between the host and pathogens in urinary tract infections, basic research has played a crucial role. This research has shed light on the pathophysiology of urinary tract infections and the impact of these interactions. Moreover, efforts are currently underway to translate this knowledge into new clinical therapies for urinary tract infections. By utilizing this understanding of host-pathogen interactions, it is hoped that new and effective treatments for urinary tract infections can be developed to alleviate the burden caused by these infections.

The clinical presentation of UTIs can vary, ranging from uncomplicated cases of UTI to more complicated cases known as UTIs. However, the majority of UTIs are typically treated empirically, without the need for specific identification of the causative agent. Bacteria are the primary microorganisms responsible for causing these infections, although fungi and viruses, although less common, have also been reported as causative agents in some instances. In addition to these microorganisms, other pathogenic microbes such as; *Klebsiella pneumoniae*, *Aspergillus chimera*, *Enterococcus faecalis*, and *Staphylococcus spp.* contribute to the development of severe urinary tract infections (*Wartu J. R et al., 2019*).

One of the major challenges in the management of UTIs is the increasing prevalence of multi-drug resistance (MDR) among the causative organisms. This rise in MDR not only hampers the efficacy of antibiotic treatment but also contributes to the spread of antibiotic resistance, further complicating the management of UTIs. The emergence of increased resistance in uremic pathogens poses a significant threat to public health, as it not only limits the treatment options available but also imposes a substantial economic burden associated with the management of UTIs. Therefore, it is crucial to explore the different factors associated with UTIs, including the pathogenic mechanisms employed by the causative microorganisms, to develop effective strategies for prevention and treatment.

During their study at Kathmandu Model Hospital, *Pankaj Baral et al., 2012*, collected 219 bacterial isolates

from 710 urine samples. The researchers employed strict standard laboratory procedures to analyze the samples and isolates. Among the significant bacterial growths, 30.8% of the isolates were found to be multi-drug resistant (MDR). The most commonly isolated organism was *E. coli*, accounting for 81.3% of the isolates. Of these *E. coli* isolates, 38.2% were found to be multi-drug resistant. The second most common organism, *Citrobacter spp*, exhibited an even higher rate of multi-drug resistance (72.7%). Notably, the production of extended-spectrum beta-lactamase (ESBL), an enzyme that confers resistance to a broad range of beta-lactam antibiotics, was detected in 55.2% of the subset of *E. coli* multi-drug resistant isolates. Further investigation revealed the presence of plasmids of varying sizes (ranging from 2 to 51 kb) among the 29 *E. coli* multi-drug resistant isolates, resulting in 15 different profiles. The most prevalent plasmid, measuring 32 kb, was found in all *E. coli* strains containing plasmids. In terms of transmissibility, most of the *E. coli* isolates tested for drug resistance were capable of transferring plasmid-mediated multi-drug resistant phenotypes and ESBL profiles.

In recent years, there has been a rise in the prevalence of antimicrobial resistance during the management of outpatient UTIs. Clinicians must consider specific clinical and epidemiological risk factors associated with these infections, particularly when dealing with multidrug-resistant (MDR) uropathogens. Based on the available literature, there are several key points to consider. Fosfomycin and nitrofurantoin continue to demonstrate effectiveness against most MDR *Escherichia coli* urinary tract infections. However, there has been an increase in resistance rates to trimethoprim-sulfamethoxazole worldwide, although it remains clinically effective. Beta-lactams, a class of antibiotics, exhibit high resistance rates and low clinical efficacy in the management of UTIs. Fluoroquinolones, another class of antibiotics, are not recommended as first-line agents due to their high resistance rate among urinary tract infections (*Emily Walker et al. 2016*). Therefore, it is crucial for clinicians to carefully assess the specific clinical and epidemiological factors associated with UTIs and consider alternative treatment options when dealing with multidrug-resistant uropathogens.

It was found that 62.5% of the total samples, which accounts for 125 out of 200, exhibited positive biofilm formation. Furthermore, a substantial 78% of the samples, equivalent to 156 out of 200, were categorized as multidrug-resistant (MDR). The isolates demonstrated varying degrees of resistance to different antibiotics, with the highest resistance rates observed against Trimethoprim-sulfamethoxazole and amoxicillin, both reaching a staggering 93%. Following closely behind was gentamicin, with a resistance rate of 87%. On the other hand, the lowest resistance was detected against imipenem, with a mere 0.5% resistance rate. In terms of the prevalence of neurovirulence genes,

the findings revealed that the most common neurovirulence gene was Fim, present in 53.5% of the isolates. Pap, on the other hand, was detected in 21% of the isolates. SFA was identified in 13% of the cases, while Afa was found in 8% of the cases. Cnf was observed in 5.5% of the cases, whereas the least prevalent gene was Hyl, with a presence of 0% (Paul Katongole *et al.*, 2020).

Based on the results obtained from this study, it can be concluded that there is a significant prevalence of biofilm-forming uropathogenic *E. coli* strains that are associated with the multidrug-resistant phenotype. This highlights the importance of regular monitoring of antimicrobial resistance and biofilm formation to inform appropriate antibiotic treatment for biofilm-associated urinary tract infections. It is crucial to keep a close eye on these trends and patterns to ensure effective management and control of such infections. Furthermore, the identification of the specific neurovirulence genes provides valuable insights into the virulence potential of these strains, which can aid in the development of targeted therapeutic strategies. To effectively address the challenges posed by biofilm-associated urinary tract infections, it is imperative to adopt a multidisciplinary approach that involves collaboration between clinicians, microbiologists, and researchers. This collaborative effort can facilitate the development of innovative treatment options and strategies that target the biofilm-forming strains and combat the issue of antimicrobial resistance. Additionally, it is crucial to enhance public awareness regarding the importance of preventive measures and proper hygiene practices to minimize the risk of acquiring such infections. Education campaigns and public health initiatives can play a pivotal role in promoting these practices and reducing the burden of biofilm-associated urinary tract infections. In conclusion, the findings of this study shed light on the significant prevalence of biofilm-forming uropathogenic *E. coli* strains that exhibit multidrug resistance. The high resistance rates observed against commonly used antibiotics emphasize the need for judicious antibiotic prescribing practices and the development of novel therapeutic approaches. Regular monitoring of antimicrobial resistance patterns and biofilm formation is essential to inform appropriate treatment strategies and ensure favorable patient outcomes. By addressing these challenges, healthcare professionals can effectively manage biofilm-associated urinary tract infections and reduce the associated morbidity and mortality rates.

Glenn T Werneburg (2022) studied that CAUTI, which stands for catheter-associated urinary tract infection, is the most prevalent type of infection that occurs as a result of healthcare-associated activities and is also responsible for causing secondary bloodstream infections. Despite the numerous advancements that have been made in the fields of diagnosis, prevention, and treatment, CAUTI continues to pose a significant burden on the healthcare system, with alarmingly high rates

of antibiotic resistance. This review will delve into the current paradigms and challenges associated with the management of CAUTI and subsequently shed light on the potential prospects in terms of diagnosis, prevention, and treatment. The evaluation will encompass a thorough analysis of clinical and translational evidence, as well as an examination of key basic science studies that serve as the foundation for preventive and therapeutic approaches. It is worth noting that there are several innovative diagnostic strategies and treatment decision aids currently in development, which aim to expedite the diagnosis process and enhance the accuracy and judicious use of antibiotics. These novel approaches involve the utilization of various classes of biomarkers, often in conjunction with artificial intelligence algorithms, as well as the incorporation of cell-free DNA and other cutting-edge techniques. Furthermore, researchers are actively exploring new preventive strategies, such as the development and investigation of catheter coatings and materials, vaccination protocols, and the exploration of bacterial interference. Nevertheless, the antibiotic pipeline still faces a shortage of viable options, thus necessitating the exploration of alternative strategies for the identification of new classes of antibiotics and the rational design of small molecule inhibitor substitutes, specifically tailored for the treatment of CAUTI.

Urinary tract infections, which are the most prevalent urologic afflictions in the United States and one of the most widespread bacterial infections affecting any organ system, are characterized by the persistence of biofilms within the urinary tract and on catheter surfaces. This persistence is primarily attributed to the remarkable resistance of biofilm microorganisms to both host defense mechanisms and antibiotic therapy. The initial step in the formation of biofilm infections is the adhesion of bacteria, and thus, the prevention of bacterial adhesion represents a highly promising strategy for controlling biofilms. Accumulating evidence suggests that capsular polysaccharides play a crucial role in the process of adhesion and the subsequent development of pathogenicity. Consequently, the present investigation endeavors to shed light on the intricate interplay of physiochemical and specific binding interactions during the adhesion process by focusing on the behavior of colanic acid exopolysaccharide mutant strains. To evaluate bacterial adhesion, we examined isogenic uropathogenic *Escherichia coli* strains that exhibited variations in colanic acid expression. To directly gauge the reversible physiochemical and specific binding interactions between the bacterial strains and various substrates as the bacteria initially approached the interface, the atomic force microscope (AFM) was employed. The AFM results unequivocally indicate that the repulsive forces between the colanic acid mutant strains and hydrophilic substrates cannot be attributed solely to electrostatic interactions (Andrea Hanna *et al.*, 2003). Furthermore, the involvement of hydrophobic interactions in the adhesion of the colanic acid mutant strains was deemed negligible. In addition to the AFM

force measurements, parallel-plate flow cell studies were conducted to assess adhesion and compare the results with those obtained from the AFM measurements. These complementary studies aimed to demonstrate how prolonged incubation times can affect bacterial adhesion. The findings derived from this comprehensive investigation conclusively demonstrate that the capsular polysaccharide colanic acid does not enhance bacterial adhesion. On the contrary, it impedes the establishment of both specific binding and time-dependent interactions between uropathogenic *E. coli* and inert substrates.

Biofilm formation as a different way of life for microorganisms, where they exhibit a multicellular behavior that enhances their survival in various environmental conditions. These biofilms can be found on both living and non-living surfaces, both in natural environments and in healthcare facilities. In hospitals, biofilms on vents and medical equipment serve as reservoirs for pathogens, facilitating their transmission to patients. Within a host, biofilms allow pathogens to evade innate immune defenses, resulting in long-term persistence. In this review, we present a comprehensive overview of the processes involved in biofilm formation on surfaces and within eukaryotic cells, with a focus on several medically significant pathogens. We also discuss recent advancements in novel strategies targeting biofilm prevention and dissolution. (Maria Kostakioti, 2013)

Scoparia dulcis, known as, is a type of erect herb that can be found in annual form. This herb is widely distributed across tropical and sub-tropical regions of countries such as India, America, and Brazil. The utilization of the entire *Scoparia dulcis* plant is for the treatment of various ailments including but not limited to diarrhea, stomach ache, kidney stone, and fever. The significance of *Scoparia dulcis* lies in its affordability and minimal side effects, making it a preferred choice for a large portion of the population. It has been reported by the World Health Organization (WHO) that approximately 80% of the population still heavily relies on plant-based drugs for their healthcare needs. Hence, it is crucial to investigate the antioxidant properties of different extracts derived from *Scoparia dulcis*, which belongs to the Scrophulariaceae family and is believed to possess medicinal properties (Aysha Reem T P 2022).

The antioxidant potential observed in *Scoparia dulcis* could be attributed to the presence of polyphenolic compounds. These compounds have been widely acknowledged for their ability to scavenge free radicals and protect the body against oxidative stress. To gain a deeper understanding of *Scoparia dulcis*, this review encompasses information on its morphological, phytochemical, and pharmacological aspects. By exploring these facets, we can unravel the true potential of *Scoparia dulcis* and its possible applications in the field of medicine. One notable finding from this investigation is that *Scoparia dulcis* therapy may hold promise in the management of anemia, a condition

characterized by a deficiency of red blood cells or hemoglobin in the blood. Moreover, it is plausible that *Scoparia dulcis* could also be beneficial in addressing other forms of illness and disease. In conclusion, *Scoparia dulcis*, also known as Aysha Reem T P2022, is a versatile herb that has gained substantial attention due to its therapeutic properties. Its widespread availability, minimal side effects, and reliance on plant-based drugs by a large portion of the population further accentuate the importance of studying this herb. The evaluation of different extracts derived from *Scoparia dulcis* has shed light on its antioxidant potential, which can be attributed to the presence of polyphenolic compounds. By delving into its morphological, phytochemical, and pharmacological aspects, we have gained valuable insights into the various dimensions of this herb. Ultimately, it is evident that *Scoparia dulcis* therapy could potentially be an effective approach to managing anemia and other related conditions. Further research is warranted to fully explore its capabilities and unlock its full potential in the field of medicine.

The opening of author links in the research paper serves as a gateway to further explore the work conducted by (Toshimitsu Hayashi 1996). The study focused on the scopadulciol (SDY) production in callus and multiple shoots derived from *Scoparia dulcis*, specifically an SDX-type plant. The investigation revealed that both callus and multiple shoots yielded the same amount of diterpenoid, namely scopadulciol (SDY). Interestingly, the levels of diterpenoid production in the multiple shoots were found to be five times higher in comparison to the multiple shoots obtained from an SDB-type plant. This intriguing finding suggests that the control mechanism governing diterpenoid production differs significantly between the SDX- and SDB-types, shedding light on the complex regulation of this important biochemical pathway.

This evaluation discusses the chemical elements and pharmacological consequences of *Scoparia dulcis* L. (*S. dulcis*) plants. So far, about a hundred and sixty compounds have been recognized from *S. dulcis*, amongst which one hundred fifteen compounds can be associated with the remedy of metabolic syndrome. Extracts of *S. Dulcis* have the results of lowering fasting blood glucose level, growing the Plasma insulin level, and stimulating insulin secretion to deal with diabetes. They moreover produce antihyperlipidemic results through developing serum high-density lipoprotein levels, the anti-atherogenic index of plasma, and HMG-CoA reductase activity (Zikang Jiang, Jinghui Sung 2021). The chemical composition of glutinol and glutinous, remoted from *S. dulcis*, provides potential anti-inflammatory effects. These compounds also can lessen general cholesterol, Triacylglycerol, and low-density lipoprotein (LDL)- ldl cholesterol and growth high-density lipoprotein (HDL)- cholesterol to provide the anti-atherosclerotic effect. *S. Dulcis* exerts anti-arthritis houses through its impact on cytokine degrees,

substantially lowering IFN- γ and IL-6 degrees and raising IL-10 degrees. The extracts perform hepatoprotective impact with the aid of using stopping the descent of the antioxidative enzymes of superoxide Dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GRd), and glutathione-S-transferase (GST). Therefore, *S. Dulcis* affords new ability for remedy given its several healing houses and may be promoted as a complementary or opportunity remedy for sufferers with continual conditions.

Traditional medical healers think that the commonly used medicinal herb *Scoparia dulcis* has antihypertensive properties. In this work, Wistar rats are used to test *Scoparia dulcis* antihypertensive properties. Procedures: Plant specimens were gathered from neighborhood gardens in Portharcourt and identified at the University of Portharcourt's Department of Plant Science. The plant's leaves were extracted into methanol and water. Conventional techniques were used for phytochemical screening, and the Karben Arithmetic approach was used to calculate the LD50 in acute toxicity experiments. Rats were endotracheally intubated after urethane was used to put them to sleep. Following that, the carotid artery and jugular vein of the rats were cannulated. Saponins were absent. For the methanol and aqueous, the LD50 was 450 and 650 mg/kg, respectively. For both extracts, there were notable ($P < 0.05$) sympathomimetic and hypertensive effects at dosage levels of 40, 80, and 160 mg/kg. Talk: Even though the *Scoparia dulcis* plant has been historically used to cure hypertension, scientific study has not proved this property; instead, the plant has been shown to increase BP, MAP, and HR, as the figures above suggest. Catecholamines found in the plant are responsible for these effects upon parenteral administration. Traditional medicine relies on subjective symptoms like headache, sleeplessness, and malaise to diagnose hypertension; there are no scientific methods for this, such as blood pressure monitoring. The improvement in the aforementioned symptoms in hypertension patients may be mistakenly assumed to represent the BP-reducing effects among traditional medical healers. Extracts from the *Scoparia dulcis* plant have been documented to have analgesic, anti-inflammatory, and sedative actions (C O Esume *et al.*, 2011).

The comprehensive investigation of the pharmacognostic characteristics of *Scoparia dulcis* Linn., a plant belonging to the Scrophulariaceae family. The evaluation encompasses a thorough examination of various aspects such as morphology, microscopy, physicochemical properties, and phytochemical screening. To analyze the plant's structure, transverse and longitudinal sections were prepared and subjected to staining with different reagents and dyes. This allowed for the identification and differentiation of various plant parts. Furthermore, the physicochemical constants of the entire plant, including ash value, extractive value, and moisture content was determined. Moving on to the phytochemical screening,

both aqueous and methanolic extracts obtained through maceration and soxhletion methods were examined. The results indicated the presence of several bioactive compounds such as alkaloids, glycosides, carbohydrates, phenolic compounds, flavonoids, saponins, proteins, and amino acids. By establishing these parameters, this study contributes to the authentication of *S. dulcis* and aids in distinguishing it from other species within the same genus. The information obtained from this extensive evaluation will be valuable for further research and utilization of this plant in various fields such as medicine, pharmacology, and phytotherapy (Mishra 2012).

Pain, which is commonly acknowledged as one of the primary symptoms observed in individuals suffering from knee osteoarthritis, serves as the utmost rationale behind patients seeking medical attention. To alleviate the discomfort associated with various painful conditions, the utilization of *Scoparia dulcis* has gained popularity among the general population. Hence, the objective of the present study conducted by Marcus Vinícius Viégas Lima *et al* in 2019 was to thoroughly assess the analgesic and anti-inflammatory effects of the crude extract of *S. dulcis* in an experimental model of osteoarthritis. To achieve this aim, the experiment was carried out on Wistar rats that were divided into four distinct groups, each comprising 5 animals, namely healthy, saline, crude extract, and meloxicam groups. The induction of knee osteoarthritis was accomplished via the intra-articular injection of sodium monoiodoacetate. The study encompassed several crucial steps. Initially, the clinical parameters associated with pain were meticulously evaluated on days 0, 5, 10, 15, and 20 after the induction of osteoarthritis. Secondly, the researchers meticulously appraised the potential inhibition of cyclooxygenase and quantified the cytokines present in the synovial fluid. Moreover, the scientific team conducted an in-silico test and Molecular Docking tests to further enhance their understanding of the subject matter at hand. (Viégas Lima *et al* 2019)

Additionally, a comprehensive histopathological evaluation was carried out on the articular cartilage by employing safranin O staining. The culmination of these aforementioned steps led to the emergence of several noteworthy outcomes. Specifically, it was revealed that a 15-day treatment course involving the administration of the crude extract effectively mitigated edema, spontaneous pain, peripheral nociceptive activity, and proinflammatory cytokines in the synovial fluid. Furthermore, it was observed that the highest degree of cyclooxygenase 2 inhibition in the crude extract was attained at a concentration of 50 $\mu\text{g/mL}$. Consequently, it can be concluded that the crude extract of *S. dulcis* possesses immense therapeutic potential when it comes to the treatment of osteoarthritis, primarily due to its anti-inflammatory and anti-nociceptive properties.

Urinary tract infection, is a prevalent infectious disease

that affects individuals of both genders. The etiological microbial pathogens infiltrate the urinary tract tissues, commencing from the renal cortex and extending to the urethral meatus. One of the major challenges in combating this condition lies in the high incidence of drug-resistant microbes among urinary tract infection cases. Consequently, addressing this issue and searching for effective solutions is imperative. In light of this background, a comprehensive study was conducted to investigate the epidemiology of urinary tract infection among the population of Kanyakumari District, situated in South India. (Christy VR *et al.*, 2019)

For this particular study, the researchers selected samples from suspected cases who sought clinical evaluation in a prominent clinical laboratory. Over six months, a total of 1824 cases underwent various clinical analyses to determine the presence of urinary tract infection. Among these cases, 1029 were females, while 795 were males. Upon conducting a clinical examination of the suspected cases, it was revealed that the culture test yielded positive results in samples collected from males (37.23%) and females (37.99%). The study further delved into the age-wise distribution of culture-positive cases, revealing that urinary tract infections occur across all age groups, ranging from infants as young as one-month-old to elderly individuals aged between 90 and 100 years. Notably, the prevalence of urinary tract infections was found to be particularly high in women of reproductive age and those in the post-menopausal stage. Additionally, the study discovered a correlation between urinary tract infections and diabetes mellitus in post-menopausal women, as well as in elderly males suffering from diabetes and prostate problems. Moreover, pregnant women and newly married women in the age group of 21-30 were identified as being more susceptible to urinary tract infections.

In addition to the aforementioned findings, the study also shed light on the occurrence of pediatric urinary tract infections in both genders. Several factors were identified as predisposing individuals to urinary tract infections in the study area. These factors include lifestyle changes, poor personal hygiene practices, nutritional deficiencies, catheter use, unclean baby napkins, and immune deficiencies. Furthermore, the analysis of urinary tract infection-positive cultures revealed the presence of various microbial pathogens, including *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Morganella morganii*, *Streptococcus species*, *Staphylococcus aureus*, *Enterococcus species*, *Proteus vulgaris*, *Chromobacterium violaceum*, *Serratia species*, as well as the fungal pathogen *Candida species*. In summary, the study conducted in the Kanyakumari District of South India shed light on the epidemiology of urinary tract infections in the region. The research revealed the high prevalence of urinary tract infections among both genders, spanning across all age groups. Furthermore, various risk factors contribute to the development of

urinary tract infections were identified, highlighting the importance of promoting good hygiene practices and addressing lifestyle and nutritional deficiencies. The presence of drug-resistant microbial pathogens further emphasizes the need for effective strategies to combat urinary tract infections in this population.

Medicinal plants have been a valuable source of diverse therapeutic agents throughout history, as highlighted by the research conducted by Prabavathy Duraisamy *et al* in 2015. Among the plethora of medicinal plants, *Scoparia dulcis* stands out as a commonly found herb in India, renowned for its remarkable potential in the treatment of diabetes. While the plant has traditionally been utilized for diabetes management, it also possesses noteworthy antibacterial properties. In the present study, the researchers aimed to investigate the impact of *S. dulcis* extract on two major urinary tract infections (UTIs) causing bacteria, namely *Escherichia coli* and *Staphylococcus aureus*. To begin the investigation, the researchers utilized ethanol to extract the bioactive compounds from the plant. The focus of the study was to analyze the effect of the plant extract on various virulence factors associated with the pathogenicity of uropathogens. Specifically, the researchers examined hemolysis, haemagglutination, proteolysis, lipolysis, cell surface hydrophobicity, and gelatinase production as key indicators of virulence. It was observed that the extract exhibited potent inhibitory effects on almost all the virulence factors of both *E. coli* and *S. aureus*.

Consequently, the study's results suggest that *Scoparia dulcis* acts as a potent inhibitor of both bacterial strains, effectively targeting their virulent factors involved in hemolysis, haemagglutination, proteolysis, lipolysis, and gelatinase production. These findings hold significant promise for the development of novel therapeutic strategies against urinary tract infections, particularly those caused by *E. coli* and *S. aureus*. Moving forward, it is crucial to expand the scope of this study and include in vivo experiments. By conducting in vivo studies, researchers will be able to evaluate the efficacy of the plant extract more holistically and determine its potential as a commercial drug for the treatment of urinary tract infections. Such future investigations will provide invaluable insights into the practical application of *Scoparia dulcis* and its potential role in combating this prevalent and challenging medical condition.

3. AIM AND OBJECTIVES

3.1 Aim

Isolation and Screening of uropathogens, phytochemical Analysis and Antibacterial effect of *Scoparia dulcis* on uropathogens.

3.2 Objective

- Isolation and identification of the uropathogens.
- Screening of isolates.
- Phytochemical analysis of *Scoparia dulcis*.
- Antimicrobial activity of extracts of *Scoparia dulcis*

on uropathogens.

- FTIR analysis
- GCMS analysis
- Anti-oxidant property

Work plan

- 1) Collection of urine sample.
- 2) Isolation and purification of uropathogenic bacteria.
- 3) Biochemical characterization of the isolates.
- 4) Screening of isolated bacterial species (screening for biofilm formers).
- 5) Collection of leaf of plant *scoparia dulcis*.
- 6) Preparation of leaf extracts.
- 7) Phytochemical analysis.
- 8) Anti bacterila test (well diffusion).
- 9) Ftir analysis
- 10) Gcms analysis
- 11) Anti oxidant property

4. MATERIALS AND METHODS

4.1 Collection of urine samples

Morning midstream urine was collected from patients with UTI in a sterile leakproof container. About 20 ml of samples were collected. The sample was delivered to the laboratory within an hour.

4.2 Confirmation of infection in the urine sample

The collected urine sample is directly inoculated into blood agar plates by a calibrated loop method to check whether the urine sample is infected or not.

4.3 Differentiation of uropathogens

Hichrome UTI agar is used as a differential media for the isolation of urinary tract infection-causing bacteria. In this agar media, we can see different colors and morphology for different species. Thus, we can differentiate the uropathogens.

The media can be made by suspending 32.45g of Hichrome UTI agar to 1000 ml distilled water. A sufficient amount of agar is added. The prepared solution is heated to dissolve agar in the media prepared. Then the media was autoclaved the media at 121 degrees for 15mins and 15 lbs. The media is poured and allowed to solidify.

The *Enterococcus faecalis* forms blue and small colonies. Where, *Escherichia coli* forms; Pink- purple colonies, *Klebsiella pneumoniae* seemed to be Blue to purple- mucoid, as well as *Pseudomonas aeruginosa* forms Colorless or greenish pigment may be observed on the colony-formed area. Also, *Proteus mirabilis* forms light brown. Finally, *Staphylococcus aureus* is Golden Yellow.

4.4 Isolation and purification of uropathogens

4.4.1 EMB agar

Eosin Methylene Blue the selective media for the identification of gram-negative bacteria, especially *E. coli*. was prepared. EMB agar medium was heated to

dissolve media completely. It was Sterilized by autoclaving at 121°C for 15 minutes. The media was cooled to hand bearing hot. Then it was poured the media into sterile Petri plates and allow it to solidify. Inoculate the culture as a quadrant streak and incubated the plate at 37°C for 24 hours. The *E. coli* colonies were observed as a green metallic sheen after incubation.

4.4.2 Cetrimide agar

The cetrimide agar medium was prepared for the isolation of *P.aeruginosa*. The media was sterilized by autoclaving at 121°C for 15 minutes. The media was cooled to hand bearing hot. The media was transferred into sterile petri plates and allow it to solidify. Inoculated as a quadrant streak and the plate was incubated at 37°C for 24 hours. The blue- green colored colonies of *Pseudomonas aeruginosa* were observed after incubation.

4.4.3 Blood agar

Blood agar is a differential medium that detects and differentiates hemolytic bacteria, particularly *Streptococcus* species. Prepare blood agar media and heat to dissolve the media completely. Sterilize the media by autoclaving at 121°C for 15 minutes. The media was cooled to hand bearing hot. Transfer the media into sterile Petriplates and allow it to solidify. And performed a quadrant streak and incubated the plate at 37°C for 24 hours. Greenish hemolysis colonies indicate the presence of *Streptococcus* after incubation.

4.4.4 Mannitol salt agar

Mannitol Salt Agar (MSA) is used as a selective medium for the isolation of pathogenic *Staphylococcus aureus*. The MSA medium is prepared and heated to dissolve the media completely. Sterilize the media by autoclaving at 121°C for 15 minutes. The media was cooled to hand bearing hot. Pour the media into a sterile petri plate and allow it to solidify. Perform a quadrant streak and incubate the plate at 37°C for 24 hours. The formation of yellow-colored colonies indicates the presence of *Staphylococcus aureus* after incubation.

4.4.5 Nutrient Agar

Nutrient agar is a general-purpose medium that supports the growth of a wide range of non-fibrous organisms. The nutrient agar medium is prepared and heated to dissolve the media completely. Sterilized the media by autoclaving at 121°C for 15 minutes. The media was cooled to hand bearing hot. Pour the media into sterile Petri plates and allow it to solidify. Inoculate the culture as a quadrant streak and incubate the plate at 37°C for 24 hours. The circular colony of this bacteria is rough, opaque, fuzzy white or slightly yellow with jagged edges and was identified as *Bacillus subtilis* (Zhenxiang LU *et al*, 2018), and the *Enterococcus fecalis* were observed as mucoid colonies (*Atlas of Oral Microbiology // Subgingival Microbes*, 2015).

4.5 Characterization of bacterial isolates

The bacterial isolates were characterized using Bergey's

manual of determinative bacteriology (Holt *et al.*, 1993) by their Morphology, Microscopic, Macroscopic, Biochemical, and Physiological characteristics.

4.5.1 Microscopic examination

Bacterial isolates were aseptically inoculated into Nutrient broth. The tubes were incubated at 37°C for 24 hours. After incubation gram staining and motility test were performed.

4.5.1.1 Gram staining

The most important differential staining technique used in bacteriology was Gram's staining named after Christian Gram. The staining is performed to differentiate the Gram-positive and Gram-negative bacteria. The gram staining procedure involved the usage of important dyes such as; crystal violet (the Primary Stain), gram iodine (mordant), the decolorizer – alcohol (95%), and then safranin (the Secondary stain).

A thin smear of 24-hours-old cultures was prepared on a glass slide. The smear was allowed to air dry and fixed with heat. The slide was placed on the slide rack for staining. The smear with crystal violet- and allowed to for 30 seconds to one minute. The smear was flooded with running tap water. The smear was smeared with gram's iodine solution for 60 seconds. The excess amount of iodine solution was washed with 95% ethyl alcohol. Then the smear finally counter-stain safranin and wait for 30 seconds. The slide was washed with water and air dry to observe the slide under low- power and then on high-power objectives of the compound microscope (Rajan, 2010).

4.5.1.2 Motility test

A clean glass slide was taken and placed on the table with the uppermost depression. In the motility test hanging drop method was carried out to find out the motility of bacteria. A little vaseline or petroleum jelly was applied around the four corners of the coverslip using a match stick. A loop full of culture was transferred into the center of the coverslip. Then the cavity was placed facing down so that the depression covered the suspension. It was then pressed gently to form a seal between the coverslip and the slide. Then the slide was examined under the microscope (Rajan, 2010).

4.5.2 Biochemical characterization

The biochemical characterization was used for the primary characterization of the bacterial isolates. This can help in the easy classification of bacterial isolates at the genus level.

4.5.2.1 Indole test

The nutrient broth with tryptophan was sterilized and cooled to inoculate the culture. The culture-inoculated tubes were incubated at 37°C for 24hrs and Kovac's reagent was added to obtain a cheery red ring in the top of the culture media indicating a positive result.

4.5.2.2 Methyl Red

The methyl red test was used to detect the fermentation of glucose to stable Acid end products (often referred to as mixed acid fermentation). The chemical indicator, methyl red, is used to detect this lower pH. Methyl red is red at a pH of less than 4.4 and yellow at a pH higher than 6.0.

The nutrient broth was prepared and sterilized at autoclave, the culture was inoculated and incubated at 37°C for 24hrs, methyl red indicator was added, and the color change was observed.

4.5.2.3 Voges-Proskauer Test

Voges-Proskauer test the broth includes peptones, glucose, and a phosphate buffer. Some bacteria ferment glucose to pyruvic acid to butanediol fermentation. The Voges Proskauer test can detect acetone and diacetyl which are always present with butanediol. Therefore, the Voges-Proskauer test indirectly detects butanediol. The acetone and detected by alpha-naphthol and potassium hydroxide reagents.

The nutrient broth was prepared and sterilized at autoclave, the culture was inoculated and incubated at 37°C for 24hrs, Barritt's reagent was added, and the color change was observed.

4.5.2.4 Citrate utilization test

The cultures were inoculated into Simmons Citrate agar slants and incubated for 24 hours at 37°C. After incubation, the tubes were observed for growth and color change.

4.5.2.5 Triple sugar ion test

Growth of the organism on the TSI slant can indicate the type of sugar fermented and in addition, identifying the hydrogen sulfide producer with acid production the color of phenol red indicator turns yellow. An alkaline reaction of the medium was indicated by the purple color and the production of hydrogen sulfide was indicated by the formation of a black color as hydrogen sulfide combines with ferrous ammonium sulfate.

TSI medium was prepared and dispensed 5ml into the test tubes sterilized and allowed to solidify in the slant position. A loop full of culture was streaked on the slant and incubated at 37°C for 24 hours. The appearance of a change of yellow indicated the positive result for gas from an orange color to red or yellow indicated a positive result for gas production.

4.5.2.6 Catalase test

Most aerobic organisms are capable of splitting hydrogen peroxide to release free oxygen. The release of free oxygen can be seen readily by the white bubble in a few drops of hydrogen peroxide added to the cultures. One or two drops of 10% hydrogen peroxide were added to colonies of nutrient agar and the results were recorded.

4.5.2.7 Oxidase test

An oxidase test was done to identify whether the bacteria is producing an enzyme called oxidase. A nutrient agar media was prepared and sterilized and the culture was streaked and incubated at 37°C, the disc having the reagent tetraethyl para phenyl diamine was placed on the grown colonies, and the color change of the disc within 15 seconds. The appearance of blue color indicates the presence of oxidase enzyme.

Oxidase enzyme plays a vital role in the operation of the electron transport system during aerobic respiration. Cytochrome oxidase catalyzes the oxidation of reduced cytochrome by molecular oxygen resulting in water and hydrogen peroxide formation.

4.5.2.8 Starch hydrolysis

Prepared and sterilized starch agar medium was poured into sterile petri plates. Streak the plates individually with the given bacterial cultures. Incubate the inoculated plates at 37°C for 24 hours. Following the incubation period, flood the iodine solution over the entire starch agar surface. Examine the plates for the presence or absence of the blue-black color surrounding each test organism. Record the results.

4.5.2.9 Gelatin hydrolysis

Prepared nutrient gelatin medium and sterilized at 121°C for 15 minutes. Streak the given bacterial culture on the surface of the medium as a single-line streak. Incubated plates at 37°C for 24 hours. Following incubation flood the gelatin precipitating reagent (mercuric chloride). A clear zone around the bacterial colony indicates positive results.

4.5.2.10 Carbohydrate fermentation

Prepare broth media by combining all components in 1000 mL of distilled or deionized water and gently heating it to aid in dissolving. Select a single carbohydrate source according to your specific needs. Dispense 4-5 ml of phenol red carbohydrate broth into 13 test tubes measuring 100 mm x 13 mm. Introduce a Durham tube into each tube to facilitate the detection of gas production. Utilize a sterile inoculating loop to transfer a distinct colony from a fresh culture (aged 18 to 24 hours) of the bacterial sample and introduce it into the broth. Allow the tubes to incubate at a temperature of 35±2°C for a period of 18 to 24 hours. Monitor the broth for any alterations in color and observe the presence of trapped air bubbles within the Durham tube.

4.5.2.11 Casein hydrolysis

Prepare skim milk medium of casein medium. Sterilize at 121°C for 10 minutes. Inoculate the test organism into the plate as a single line streak. Incubate at 37°C for 24 hours. Observe for zone around the colonies.

4.6 Screening for biofilm formation

The isolates were inoculated on the sterilized and cooled media Luria broth and incubated at 37°C for 72 hours.

After incubation, the broth was discarded, and 3 ml of 70% PBS was added to each test tube. Rinse the tubes with PBS solution and they were discarded. Tubes are placed inverted for a few minutes for drying. After 15 minutes, 2 ml of 1% of crystal violet was added for 10 sec and discarded. Tubes were rinsed with distilled water 2 times to remove excess crystal violet. 3 ml of 30% glacial acetic acid was added to each test tube. Incubate for 15 mins. Absorbance was read at 540nm.

4.7 Plant sample collection

The plant leaf was collected from the wetlands of Amaravila, and Ottashekaramangalam. *Scoparia dulcis* plant of healthy disease and free leaves were selected. These leaves were washed with distilled water and rinsed in it. Then leaves were washed using tween 20 detergent for the removal of surface pollutants and contaminants. By using sodium hypochlorite (1%) the surface sterilization was done and again rinsed with distilled water.

The sterilized leaves were shade-dried at room temperature for a few days. After drying the leaves were powdered and stored in an air-tight container, in a cool, dark, and dry environment for further studies (Nivedhitha *et al.*, 2015).

4.8 Preparation of leaf extracts

The extract was prepared using polar, non-polar, and neutral solutions, i.e., ethanol, toluene, and distilled water. The dried leaf of *S. dulcis* of 0.2g/ml was soaked in the solutions taken. They are incubated at room temperature for 3 days and filtrated using Whatman no: 1 filter paper. The solvents were stored in a capped test tube for future study.

4.9 Phytochemical analysis

4.9.1 Carbohydrate

Benedict test: Take 1ml of extract and 5ml benedict reagent added and boil for 5mins in water bath. The development of bluish green/green to brick red color indicates the presence of carbohydrate

4.9.2 Coumarins

Take 2 ml of plant extract and add 3 ml of 10% NaOH followed by chloroform.

The formation of yellow color indicates the presence of coumarins

4.9.3 Flavonoids

To 2ml of plant extract add a few drops of 10% lead acetate solution. Formation of yellow and precipitate indicates the presence of flavonoids.

4.9.4 Glycosides

Kellerkilliani test: To 2ml of the extract add 1ml of glacial acetic acid and concentrated H₂SO₄. The formation of a blue color indicates the presence of glycosides.

4.9.5 Phenols

To 5ml of plant extract add 1ml of ammonium hydroxide solution and a few drops of 5% ferric chloride solution. The formation of a violet color indicates the presence of phenols.

4.9.6 Proteins

To 2ml of plant extract add 1ml of 40% NaOH and a few drops of 1% CuSO₄. The formation of a violet color indicates the presence of peptide linkage molecule in the sample extract.

4.9.7 Saponins

To 1ml of the extract add 5ml of distilled water and shake vigorously. The formation of foam and saliva after 15 minutes indicates the presence of saponins.

4.9.8 Steroids

2ml of extract dissolved with 10 ml of chloroform equal volume of concentrated H₂SO₄ along the side of the test tube. Steroids were confirmed by the change in the upper layer of the solution as red and the H₂SO₄ layer as yellow with green fluorescence.

4.9.9 Tannin

Braemer's test: To 2ml of plant extract about 2 to 3 drops of ferric chloride solution was added. The formation of green black color indicates the presence of tannins.

4.9.10 Terpenoids

Salkowski test: 1ml of the extract was added to 2ml of chloroform and concentrated H₂SO₄ along the sides. The formation of a reddish-brown ring indicates the presence of terpenoids.

4.10 Antimicrobial activity

Antimicrobial activity is the process of killing or inhibiting the disease-causing microbes. Antimicrobials may be anti-bacterial, anti-fungal, or antiviral. They all have different modes of action by which they act to suppress the infection. The agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extract. Here we use the Muller Hinton agar medium for the good diffusion method.

Prepare the Muller Hinton agar and sterilize the media by autoclaving at 121°C for 15 minutes. The media is cooled to 50°C. Pour the media into sterile Petri plates and allow it to solidify. Spread a volume of microbial inoculum over the entire surface of the agar plate. Six microbial cultures were used such as *Bacillus subtilis*, *Pseudomonas*, *E. coli*, *Streptococci* spp, *Staphylococci* spp, and *Enterococci* spp. Rest the plates for 2 minutes to get absorbed. The wells were cut into the surface of agar using a sterile well digger. Then the wells are filled with plant extracts such as distilled water, toluene, and ethanolic extract at a concentration of 200 µg. Incubate the plates at 37°C for 24hrs and the zone of inhibition was measured in mm.

4.11 Fourier Transform Infrared Spectroscopy (FTIR)

The FT-IR analysis of the sample was done using Happgenzel with spectral range 4000-650 of resolution 2cm⁻¹.

$R_f = \text{Distance traveled by solute} / \text{Distance traveled by solvent}$

4.12 Gas Chromatography and Mass spectroscopy analysis

The analysis of the volatile compound in *S. dulcis* extracts was conducted using an Agilent 8890 system equipped with an AOC-20i autosampler and a Gas chromatography-mass spectrometer (GC-MS) fitted with an Elite-5MS capillary column. For the GC-MS analysis, an electron ionization system operating in electron stimulus mode with an ionization energy of 70 eV was used. Helium gas was employed as the carrier gas at a constant flow rate of 1.2 ml/min, and an injection volume of 1 µl was utilized with a split ratio of 15:1. The injector temperature was maintained at 250°C, the ion-source temperature was set at 230°C, and the oven temperature was programmed to increase from 350°C at a rate of 5°C/min to 180°C for 3 minutes, followed by a further increase of 59°C/min to 300°C for 5 minutes. Mass spectra were acquired at 70 eV with a scan interval of 0.5 seconds and fragments ranging from 45 to 450 Da. A solvent delay of 3 minutes was employed, resulting in a total GC/MS running time of 53.5 minutes. The relative abundance of each constituent was determined by comparing its average peak area to the total areas. The mass detector used in this study was the Turbo-Mass Gold-Parkin Elmer, and the software utilized for managing mass spectra and chromatograms was Tube Mass ver-5.2.28-29.

4.13 Anti-oxidant property analysis

Preparation of crude plant extract

To extract, 3mg of dry powder (*S. dulcis*), 30 mL of water was used. The mixture was then incubated in a water bath at 40°C for 2 hours. After that, the mixture was filtered using Whatman filter paper and dried using a vacuum rotary evaporator. The dried extract was then placed in a sample bottle and kept in a dark place at -20°C until further use.

Test for antioxidant activity: DPPH Scavenging activity

Chemicals: DPPH [1, 1-Diphenyl, 2-picryl-hydrazyl], Methanol, Distilled water.

DPPH Solution Preparation: (0.004% w/v) Dissolved 0.004gm of DPPH in 100ml of 95% methanol.

Preparation of Standard and Extract: (mg/ml) Dissolved 5mg of the Standard (Ascorbic acid and extract in 5ml of distilled water to obtain a solution with a final concentration of 1000 µg/ml.

From this solution pipette out 1ml solution & mixed with 10ml of particular solution (100 µg/ml). From this solution, different concentrations are prepared.

Procedure: The free radical scavenging capacity of the extracts was determined using DPPH (Braca *et al.*, 2001). DPPH solution (0.004% w/v) was prepared in 95% methanol. The extract was mixed with the corresponding solvent to prepare the stock solution (1mg/ml). Freshly prepared DPPH solution (0.004% w/v) was taken in test tubes and sample extract was added followed by serial dilutions to every test tube so that the final volume was 3 ml, after 10 min, the absorbance was read at 515 nm using a spectrophotometer (Jasco UV-visible spectrophotometer). Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration (1mg/ml). A control sample was prepared to contain the same volume without any extract and reference ascorbic acid. The particular solvent was served as blank. % scavenging of the DPPH free radical was measured by using the following equation.

$$\% \text{ Inhibition} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100$$

The inhibition curve was plotted for triplicate experiments and represented as % of mean inhibition \pm standard deviation. IC₅₀ values were obtained by Linear regression Method.

5. RESULT

5.1 Confirmation of infection in the urine sample

During the comprehensive analysis of a substantial number of 50 urine samples that were carefully collected from RGCB lab Neyyattinkara. It was observed that a noteworthy 18 of these samples exhibited the presence of bacteria through the successful cultivation of microorganisms. The thorough examination of the physical attributes of the urine specimens encompassed an evaluation of factors such as pH levels, coloration, odor, and overall appearance. To further investigate bacterial growth, a urine culture was conducted utilizing the calibrated loop technique on blood agar, a medium optimized for cultivating microorganisms. After the completion of this procedure, the colonies that manifested on the blood agar plate were meticulously counted and determined to be approximately 10,000 colony-forming units per milliliter, a significant indication of bacterial presence and proliferation.

5.2 Differentiation of uropathogens

Hi-chrome UTI agar which is the differential medium for the identification and confirmation of uropathogens. Different chromogenic colonies were isolated and observed on this agar media. The possible pathogens observed are *Enterococcus fecalis*, *Bacillus subtilis*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*, and *E. coli*. The colony morphology and the colony color appearance on the media has been mentioned in the table 1.

5.3 Purification of uropathogens

The purification of the isolated colonies was carried out by employing specific culture media, such as Eosin

methylene blue agar for the isolation of *E. coli* and Mannitol salt agar for the isolation of *Staphylococcus* spp., in addition to Blood agar for the identification of *Staphylococcus* spp. Furthermore, the purification process also involved the utilization of Cetrimide agar for the isolation of *Pseudomonas* spp. In the case of *Bacillus subtilis* and *Enterococcus fecalis*, nutrient agar was used for purification purposes.

5.4 Characterization of bacterial isolates

Escherichia coli is a Gram-negative bacterium. Considered as rod shaped and is non-sporulating and motile organism cultured on EMB agar, *E. coli* forms colonies with a distinctive green metallic sheen. This bacterium has been found to exhibit positive results for indole, catalase, methyl red, and nitrate tests while yielding negative results for VP, citrate, and urease tests.

Staphylococcus aureus are Gram-positive cocci found in clusters, and they are non-motile, non-capsulated, and non-sporulating. They have been observed to test negative for indole, methyl red, VP, citrate utilization, triple sugar ion, oxidase, urease, and nitrate, but positive for catalase, gelatin hydrolysis, and casein hydrolysis.

Streptococcus species are Gram-positive, beta-hemolytic cocci-shaped bacteria that are nonmotile. They exhibit negative results for VP, citrate, oxidase, and methyl red tests while showing a positive response in the catalase test.

Pseudomonas, another Gram-negative bacterium, is rod-shaped and motile. On cetrimide agar, it displays a fluorescent pigment. The colonies of *Pseudomonas* on cetrimide agar are small, round, and entirely pigmented. This bacterium tests negative for indole, Voges Proskauer, urease, and oxidase, but positive for catalase, citrate utilization, methyl red, and nitrate.

Bacillus subtilis is a Gram-positive rod-shaped bacterium with a rough, opaque, fuzzy white colony featuring jagged edges. It is motile and gives positive results for VP, citrate, and catalase tests while testing negative for indole, methyl red, and oxidase.

Enterococcus fecalis is characterized by small spherical and smooth colonies, and it is Gram-positive, cocci-shaped, and nonmotile. It shows positive results for VP and carbohydrate fermentation tests while yielding negative results for indole, citrate, catalase, and oxidase tests.

The selected and purified six bacterial isolates were *Streptococcus sp.*, *Staphylococcus sp.*, *Pseudomonas sp.*, *Escherichia coli*, *Bacillus subtilis*, and *Enterococcus fecalis* based on their macroscopic, microscopic, biochemical, and physiological characteristics in comparison with Bergey's Manual of Determinative Bacteriology 9th edition. The selected isolates were tabulated (Table No. 2).

5.5 Biofilm screening

After conducting the crystal violet tube assay and subsequently measuring the OD value at 540nm using a calorimeter, the resulting absorbance values for various bacterial species were obtained. These values were found to be as follows: *Bacillus subtilis* exhibited an absorbance value of 1.26, *Staphylococcus* displayed a value of 1.09, *E. coli* demonstrated an absorbance of 0.98, *Pseudomonas* exhibited a value of 0.85, *Enterococcus* displayed an absorbance of 0.84, and *Streptococcus* exhibited the lowest value at 0.69. The result is tabulated in Table 4.

5.6 Phytochemical analysis

The plant extracts, namely the ethanolic extract, toluene extract, and distilled water extract, were subjected to a comprehensive phytochemical analysis utilizing the aforementioned procedure. The results were noted in Table 3.

5.6.1 Test for carbohydrate

When 5ml of benedict's reagent was added and subsequent boiling, the emergence of a brick-red hue signifies the existence of carbohydrates within the sample. Positive outcomes were observed for both the distilled water and ethanolic extracts, while the toluene extract yielded a negative result.

5.6.2 Test for coumarins

3ml of 10% NaOH, was added followed by chloroform to the plant extract, led to the formation of a yellow tint, served as an indicator of coumarins presence. Positive test results were obtained across all three extracts.

5.6.3 Test for flavanoids

When 10% of lead acetate was added, it resulted in a yellow precipitate formation, indicated the presence of flavonoids; notably, all three extracts of *S. dulcis* demonstrated positive outcomes.

5.6.4 Test for glycosides

The implementation of the Kellerkilliani Test involved adding 2ml of the extract to 1ml of glacial acetic acid and concentrated H₂SO₄. The emergence of a blue color signified the presence of glycosides, with positive results observed in the toluene and ethanolic extracts, while the distilled water extract exhibited a negative outcome.

5.6.5 Test for phenols

5ml of plant extracts mixed with 1ml of Ammonium Hydroxide solution and a few drops of 5% Ferric Chloride Solution, a violet color was formed, indicated the presence of phenols. Positive test results were obtained from the ethanolic extract, whereas both the toluene and distilled water extracts displayed negative outcomes.

5.6.6 Test for proteins

2ml of plant extract was combined with 1 ml of 40%

NaOH and a few drops of 1% CuSO₄, the emergence of a violet color signaled the presence of peptide linkage molecules within the sample. All extracts showcased positive results in this assessment.

5.6.7 Test for saponins

5ml of distilled water was added to the extract, followed by vigorous shaking to induce froth formation, allows for the identification of saponins presence through the persistence of froth after 15 minutes. Positive outcomes were observed in the ethanolic and distilled water extracts, while the toluene extract yielded a negative result.

5.6.8 Test for steroids

The dissolution of the extract in 10ml of chloroform and the subsequent introduction of an equal volume of concentrated H₂SO₄ alongside the test tube revealed the confirmation of steroids by distinct color changes in the solution layers. The presence of steroids was noted in the ethanolic and toluene extracts, yet remained absent in the distilled water extract.

5.6.9 Test for tannin

Conducting Braeumer's Test, involved in adding of approximately 2-3 drops of ferric chloride solution to the extract, with a resultant green-black color signifying the presence of tannins. Notably, tannins were solely detected in the ethanolic extract.

5.6.10 Test for terpenoids

The execution of the Salkowski Test entailed the addition of 2ml of chloroform and concentrated H₂SO₄ along the sides of the test tube containing the extract, leading to the formation of a reddish-brown ring that confirmed the existence of terpenoids. The detection of terpenoids was exclusive to the ethanolic extract.

5.7 Antibacterial test (well diffusion)

The antimicrobial activity was performed to analyze the bactericidal activity of *Scoparia dulcis* on the isolated and purified uropathogens resulting in producing a clean zone of inhibition over all the isolates in different diameter measurements (in mm).

The incubated plate with *Streptococcal spp* produced a larger zone of inhibition i.e., 25mm in diameter followed by, *staphylococcus spp.* and *Pseudomonas spp.* produced a larger zone of inhibition with a diameter of 21mm. *Bacillus subtilis* of diameter of 18mm. And *E. coli* and *Enterococcus fecalis* with a diameter of 15mm respectively. The result is noted in table 5.

5.8 FTIR – analysis

FTIR analysis was performed to identify the potential functional groups existing within the specimen being examined. The FTIR spectrum derived from the *Scoparia dulcis* uncovered the existence of absorption bands at various wave numbers expressed in centimeters. The identified features of the stretching

vibrations of the C-H bond, C-O bond, C-Cl bond, N=O bend, and C-N bond indicate the presence of these specific biomolecules in the sample. It is reasonable to propose that the ongoing exploration is focused on proteins that possess similar functional groups to the extract, including amide and alkanes, which offer valuable insights into the makeup of the material under investigation (Catherine 2009).

FTIR analysis has proven to be instrumental in revealing the molecular composition of the sample, shedding light on the intricate interactions at the molecular level and providing a deeper understanding of its chemical structure. This detailed analysis aids in the interpretation of the biological significance and potential applications of the identified biomolecules within the sample, offering a comprehensive perspective on its biochemical properties and functional characteristics.

5.9 GCMS analysis

The analysis conducted using Gas Chromatography-Mass Spectrometry (GCMS) revealed that the composition of the leaf consists of a total of 189 different compounds, from which 20 compounds were carefully selected based on their significant peak values indicating high bioactive potential. The shortlisted compounds include Neophytadiene, Hexadecanoic acid methyl ester, 9,12,15-Octadecatrienoic acid methyl ester, Phytol, Naphthalene, Squalene, Dotriacontane, Betulin,

dl-alpha-Tocopherol, tetrapentacontane, Campesterol, Stigmasterol, Stearic acid-TMS, 3-O-Acetyl-6-methoxycycloartenol, Lup-20(29)-en-3-ol acetate, and Methyl cis-5,8,11,14,17-Eicosapentaenoate. These compounds exhibit diverse chemical properties and bioactive functions that make them potentially valuable for various applications in pharmaceuticals, nutraceuticals, and other related industries. Further research and analysis are warranted to fully explore the therapeutic and commercial potential of these compounds for the development of new drugs or products with enhanced bioactivity and efficacy in different fields.

5.10 Antioxidant property

The assessment of the free radical scavenging potential of the aqueous extract from *S. dulcis* leaves was conducted by employing the DPPH method. Through the utilization of this particular technique, the activity of scavenging was determined by comparing the sample under examination with the established standard, which is ascorbic acid. The results depicting the percentage of inhibition corresponding to varying concentrations were graphically represented in Figures 4 and 5, while a more detailed breakdown and analysis of this data was provided in Tables 7, 8, 9, and 10. The comprehensive presentation of these findings aids in understanding the antioxidant capabilities of the *S. dulcis* leaf extract and its potential applications in various fields.

TABLES

Table 1: Colony morphology and chromatin of different bacterial isolates in the Hi-chrome agar.

Organisms	Characteristics
<i>Enterococcus fecal</i>	Blue, small
<i>Escherichia coli</i>	Pink to purple
<i>Klebsiella pneumonia</i>	Blue to purple
<i>Pseudomonas aeruginosa</i>	Colorless (greenish pigment may be observed)
<i>Staphylococcus aureus</i>	Golden yellow
<i>Bacillus subtilis</i>	White, flat round jagged colonies

Table 2: Macroscopic characterization of bacteria from urine sample.

Test performed	<i>Escherichia coli</i>	<i>Staphylococcus spp.</i>	<i>Streptococcus spp.</i>	<i>Pseudomonas spp.</i>	<i>Bacillus subtilis</i>	<i>Enterococcus fecalis</i>
Growth in selective media	Greenish blue metallic sheen on EMB agar.	Golden yellow colony on Mannitol salt agar.	Greyish green hemolytic colony on Blood agar.	Yellow-green colonies on cetrimide agar.	—	—
Colony observation	Small circular, white colonies with entire margins.	Large mucoid yellow-pigmented colonies.	small, circular, semi-transparent, and convex, small clear zone of hemolysis	Large self-translucent, irregular, yellow to green, and round.	The circular colony of this bacteria is rough, opaque, fuzzy white or slightly yellow with jagged edges	small, spherical, smooth, opaque, creamy colonies.

Microscopic observations of bacteria from urine sample

Simple staining	Rod	Cocci	Cocci	Rod	Rod	cocci
Gram staining	Gram-negative	Garm positive	Gram-positive	Garm negative	Garm positive	Garm positive
Motility	Motile	Nonmotile	Non motile	Non-motile	Motile	Non motile

Biochemical characterization of bacteria from urine sample.

Indole	Positive	Negative	Negative	Negative	Negative	Negative	Negative
Methyl red	Positive	Positive	Negative	Negative	Negative	Negative	Positive
Voges Proskauer	Negative	Positive	Positive	Negative	Positive	Positive	Positive
Citrate	Negative	Positive	Negative	Positive	Positive	Positive	Negative
Catalase	Positive	Positive	Negative	Positive	Positive	Positive	Negative
Oxidase	Negative	Negative	Negative	Positive	Negative	Negative	Negative
Triple sugar iron	A/A	A/A	Negative	A/K	A/A	-	-
Carbohydrate fermentation	Positive						

Physiological characterization of bacteria from urine sample.

Starch hydrolysis	Negative	Negative	Negative	Negative	Positive	Discrepancies
Casein hydrolysis	Negative	Positive	Negative	Positive	-	Positive
Gelatin hydrolysis	Negative	Positive	Negative	Positive	Positive	Negative

Table 3: Phytochemical analysis of *scoparia dulcis*.

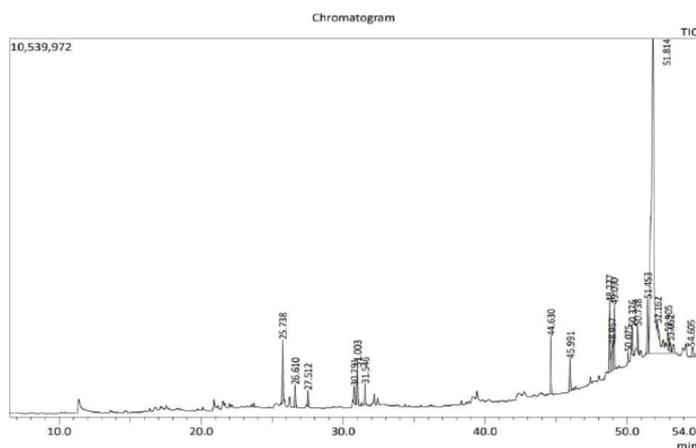
Name of test	Distilled Water	Toluene	Ethanol
Test for Carbohydrate	+	-	+
Test for Coumarins	+	+	+
Test for Flavanoids	+	+	+
Test for Glycosides	-	+	+
Test for Phenols	-	-	+
Test for Proteins	+	+	+
Test for Saponins	+	-	+
Test for Steroids	-	+	+
Test for Tannins	-	-	+
Test for Terpenoids	-	-	+

Table 4: Screening of biofilm of uropathogens isolated from urine sample.

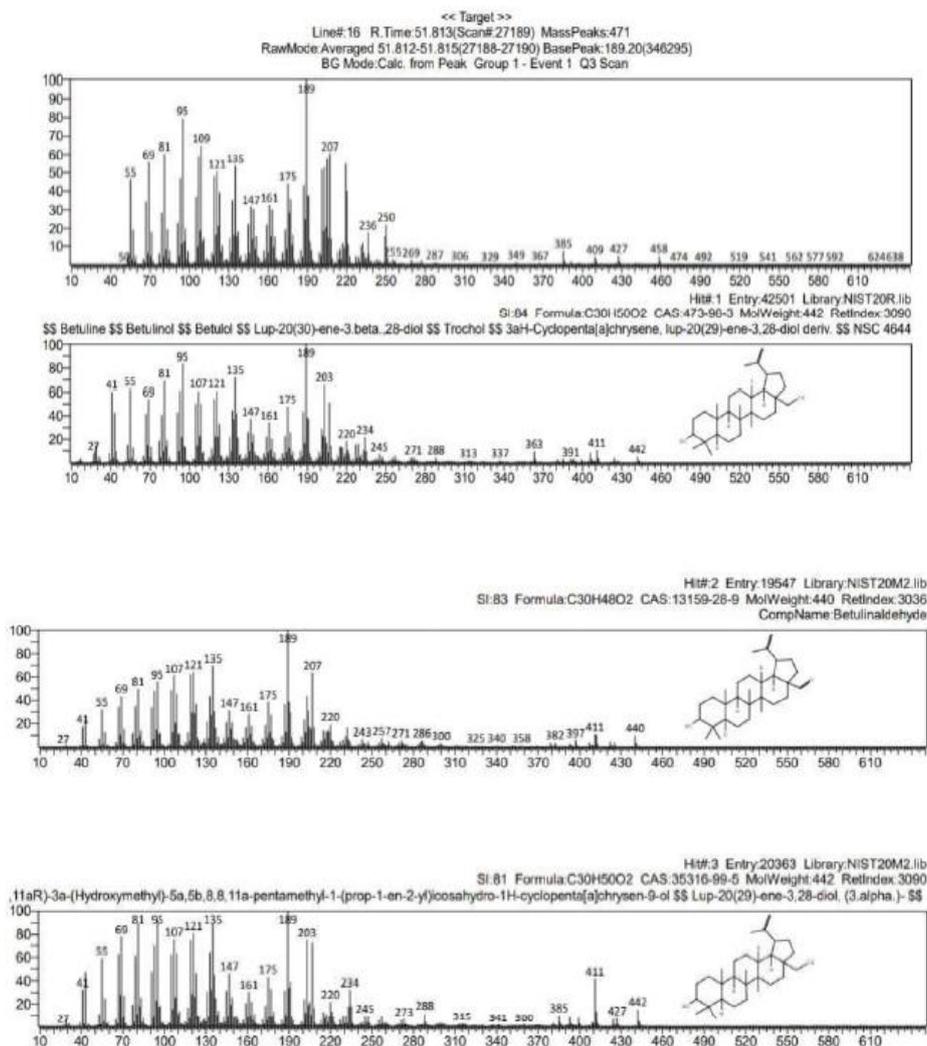
Organism	OD VALUE AT 540nm
<i>Streptococcus</i>	0.69
<i>Staphylococcus</i>	1.09
<i>Pseudomonas</i>	0.85
<i>Bacillus subtilis</i>	1.26
<i>Enterococcus fecal</i>	0.84
<i>Escherichia coli</i>	0.98

Table 5: Antibacterial test (well diffusion 200 µg) of *scopariadulcis* against bacteria isolated from urine sample.

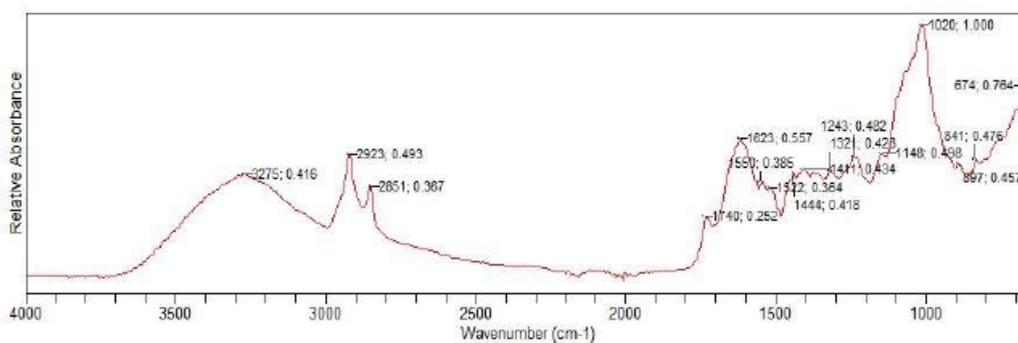
Organism	Zone of inhibition
<i>Streptococcus</i>	25mm
<i>Staphylococcus</i>	21mm
<i>Pseudomonas</i>	21mm
<i>Bacillus subtilis</i>	18mm
<i>Enterococcus fecal</i>	15mm
<i>Escherichia coli</i>	15mm

Graph 1: GCMS analysis of *scoparia dulcis*.Table 6: Bioactive compounds obtained on gcms analysis of *scoparia dulcis*.

Peak#	R.Time	Similarity	Name	CAS#	Area	Area%	Height
1	25.738	96	Neophytadiene	504-96-1	7771280	3.61	1751501
2	26.610	93	Neophytadiene	504-96-1	2874925	1.34	617028
3	27.512	95	Hexadecanoic acid, methyl ester	112-39-0	1863177	0.87	436123
4	30.791	93	9,12,15-Octadecatrienoic acid, methyl ester, (Z,; 301-0-8		2115979	0.98	479671
5	31.003	96	Phytol	150-86-7	5040607	2.34	1043782
6	31.546	82	Naphthalene, 1,2,3,4-tetrahydro-1,6,8-trimethy	30316-36-0	2642064	1.23	566336
7	44.630	95	Squalene	111-2-4	7536706	3.50	1622849
8	45.991	95	Dotriacontane	544-85-4	4216527	1.96	899398
9	48.777	94	Dotriacontane	544-85-4	9631759	4.47	1959564
10	48.957	72	Betulin	473-98-3	5781403	2.68	718397
11	49.090	93	dl- α -Tocopherol	10191-41-0	9418892	4.37	1796029
12	50.075	91	Tetrapentacontane	5856-66-6	1294167	0.60	307897
13	50.376	84	Campesterol	474-62-4	4127258	1.92	762138
14	50.738	89	Stigmasterol	83-48-7	3322883	1.54	711490
15	51.453	94	Tetrapentacontane	5856-66-6	7806332	3.63	1508036
16	51.814	84	Betulin	473-98-3	131691826	61.16	8736319
17	52.162	39	Stearic acid-TMS	57-11-4	2087872	0.97	250623
18	52.905	84	3-O-Acetyl-6-methoxy-cycloartenol	0-0-0	2849444	1.32	506539
19	53.052	76	Lup-20(29)-en-3-ol, acetate, (3 β .)-	1617-68-1	1550975	0.72	272021
20	54.605	50	Methyl cis-5,8,11,14,17-Eicosapentaenoate	10417-94-4	1701446	0.79	281017



Graph 2: The compound Beutilin-specific peak.



Graph 3: Ftir analysis of scopia dulcis.

Table 7: Antioxidant property of scopia dulcis aqueous extract.

Table 7.1: Dilution of standard ascorbic acid.

Ascorbic acid solution	Distilled water	Solution µg/ml
0.2ml	10ml	2 µg/ml
0.4ml	10ml	4 µg/ml

0.6ml	10ml	6 µg/ml
0.8ml	10ml	8 µg/ml
0.10ml	10ml	10 µg/ml
0.12ml	10ml	12 µg/ml

Table 7.2: Dilution of sample extract.

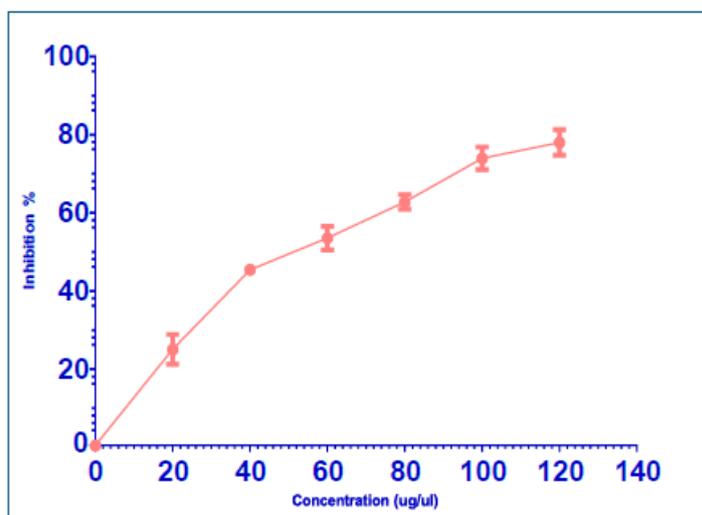
Extract solution	Particular solvent	Solution µg/ml
2.0ml	10ml	20 µg/ml
4.0ml	10ml	40 µg/ml
6.0ml	10ml	60 µg/ml
8.0ml	10ml	80 µg/ml
10.0.ml	10ml	100 µg/ml
12.0ml	10ml	120 µg/ml

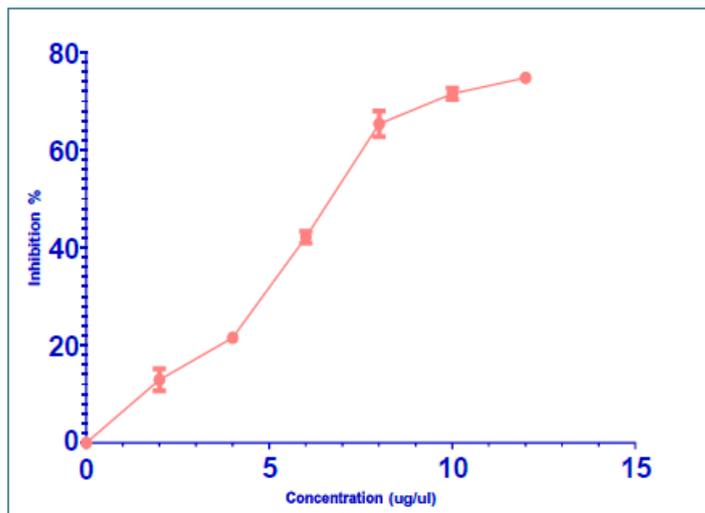
Table 7.3 DPPH radical scavenging activity of standard ascorbic acid.

S. no.	Conc. (µg/ml)	Absorbance			% Inhibition
1	Control	0.982	0.958	0.986	
2	2µg	0.865	0.824	0.859	12.92
3	4µg	0.754	0.768	0.774	21.53
4	6µg	0.568	0.574	0.551	42.14
5	8µg	0.329	0.318	0.367	65.35
6	10µg	0.265	0.281	0.287	71.53
7	12µg	0.209	0.215	0.218	74.80

Table 7.4: DPPH Radical scavenging activity of alan aqueous extract.

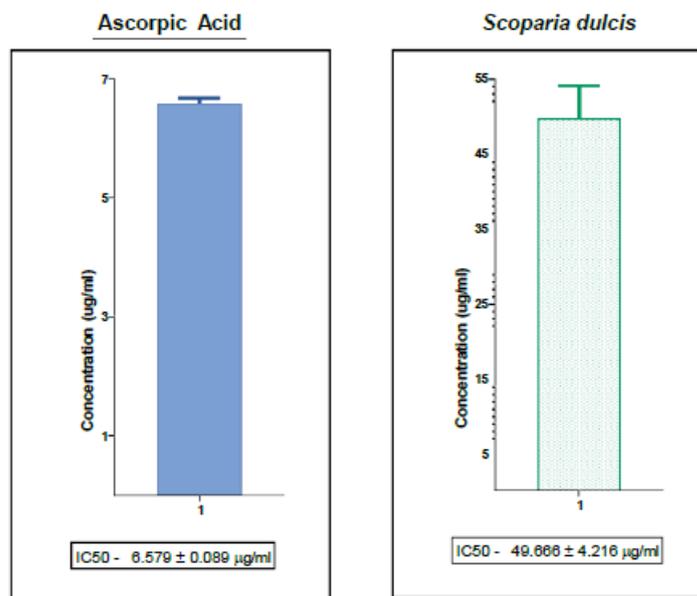
S. no.	Conc. (µg/ml)	Absorbance			% Inhibition
1	Control	0.99	0.984	0.987	-
2	20µg	0.701	0.758	0.772	24.65
3	40µg	0.53	0.546	0.552	45.02
4	60µg	0.43	0.468	0.489	53.16
5	80µg	0.389	0.352	0.372	62.41
6	100µg	0.228	0.281	0.273	73.59
7	120µg	0.203	0.258	0.201	77.64

Graph 4: DPPH Radical scavenging activity of *Scoparia dulcis* Aqueous extract.



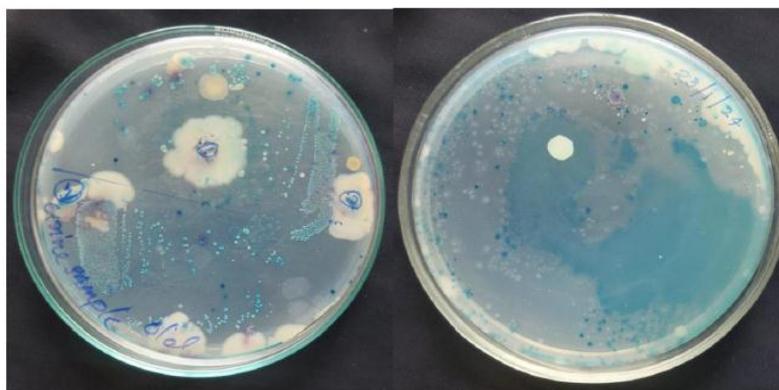
Graph 5: DPPH radical scavenging activity of standard ascorbic acid.

Comparison of the standard ascorbic acid with *scopariadulcis*



FIGURES

Different organisms in hi chrome uti agar





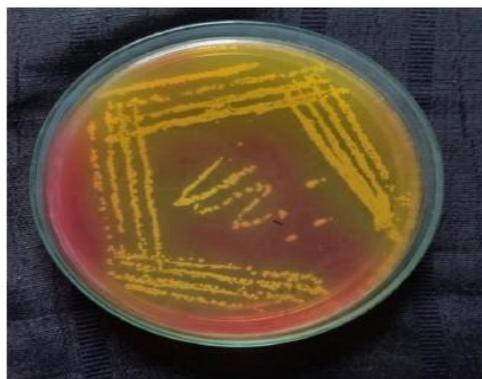
Growth of bacteria isolated from urinesample- *enterococcus* on nutrient agar



Growth of bacteria isolated from urinesample-*bacillus subtilis* on nutrient agar



Growth of bacteria isolated from urinesample -*streptococcus* on blood agar



Growth of bacteria isolated from urinesample- *staphylococcus* on mannitol salt agar

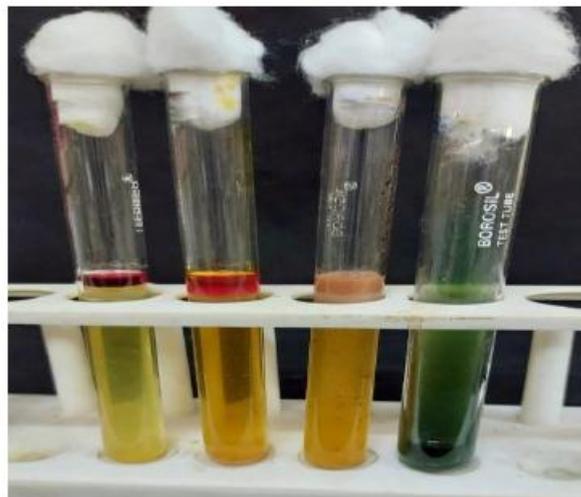


Growth of bacteria isolated from urinesample *e. coli* on emb agar

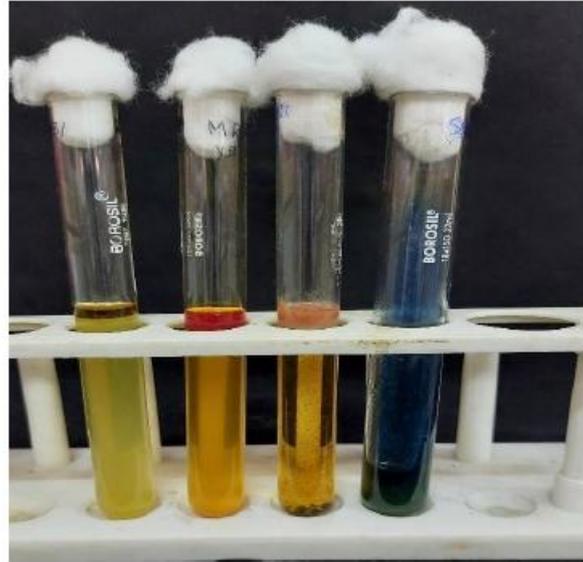


Growth of bacteria isolated from urinesample -*pseudomonas* on cetrimide agar

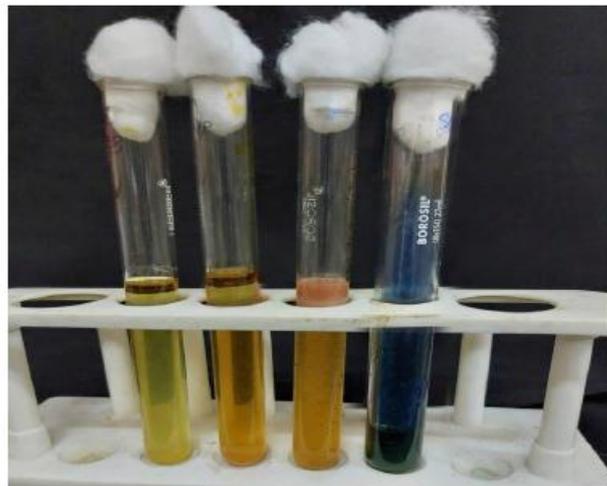
Biochemical characterization of bacteria isolated fromuropathogen



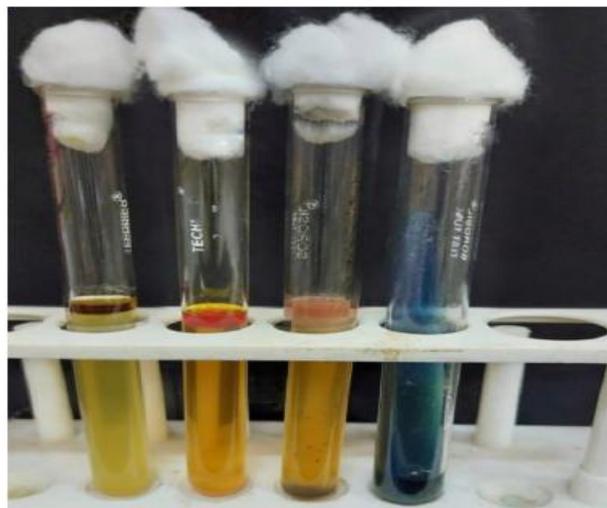
IMVIC TEST FOR *E. coli* isolated from urine sample.



IMVIC TEST FOR *Staphylococcus* isolated from urine sample.



IMVIC TEST FOR *Pseudomonas* isolated from urine sample.



IMVIC TEST FOR *Bacillus* isolated from urine sample.



IMVIC TEST FOR *Enterococcus fecalis* isolated from urine sample.



Catalase Positive

Catalase Negative



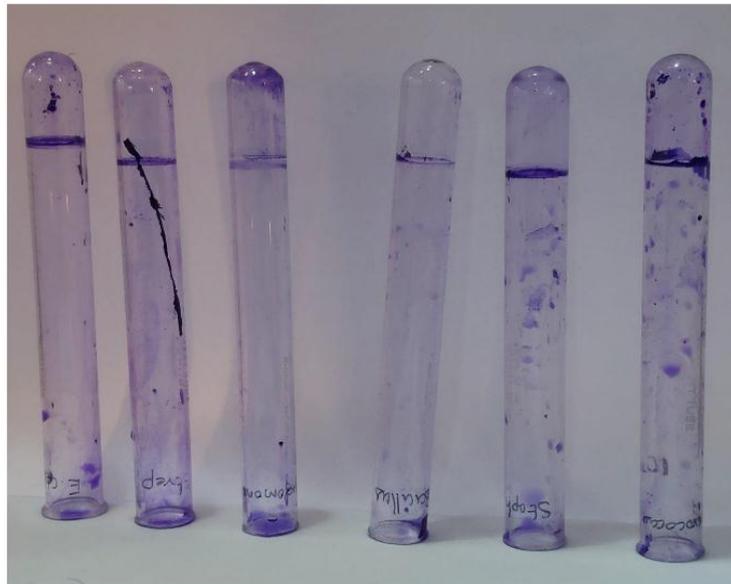
Oxidase Positive

Oxidase Negative

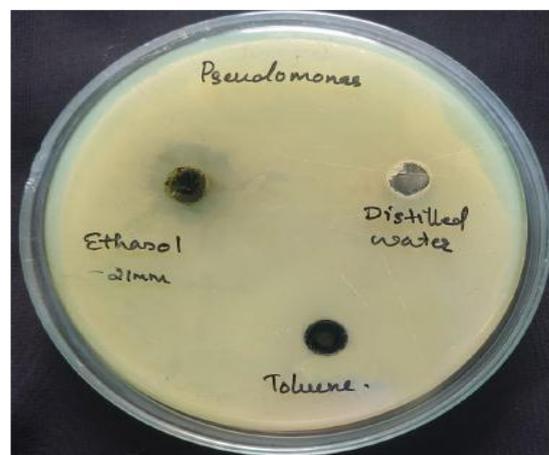
Scoparia dulcis – The plant used for the antioxidant, Phytochemical, Antibacterial screening on uropathogens



Screening of biofilm of bacteria isolated from urine sample



Antibacterial test against the bacteria isolated from urine sample by extracts of *scoparia dulcis*



Pseudomonas isolated from urine sample.



Streptococcus isolated from urine sample.



Enterococcus fecalis isolated from urine sample.



Bacillus subtilis isolated from urine sample.

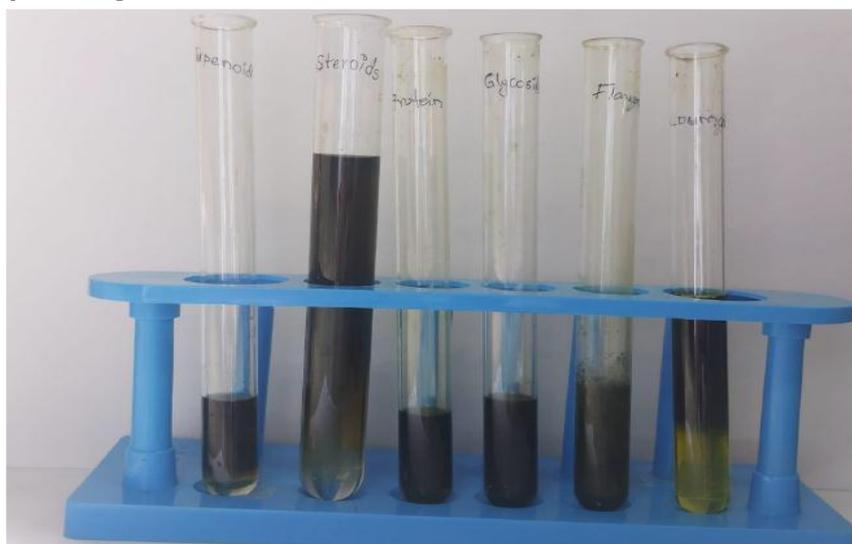


Staphylococcus isolated from urine sample.

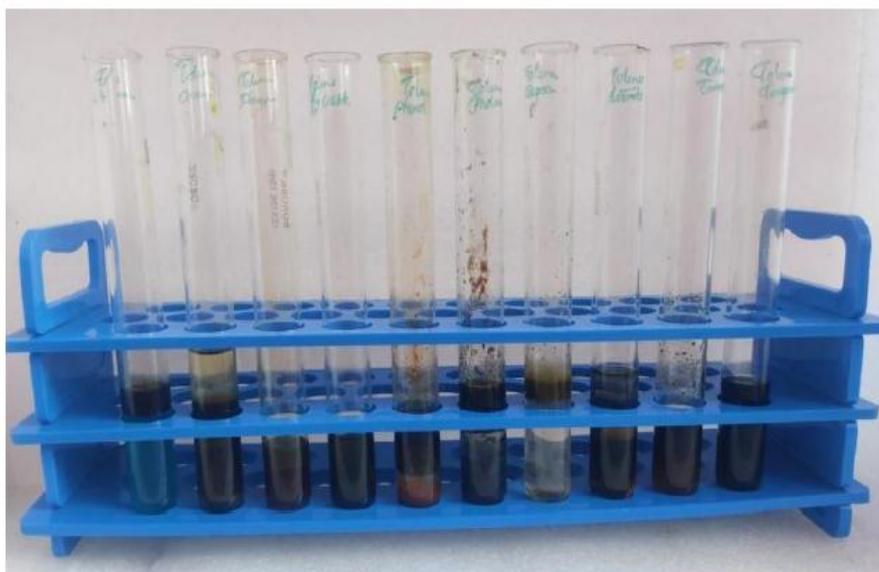


E. coli isolated from urine sample.

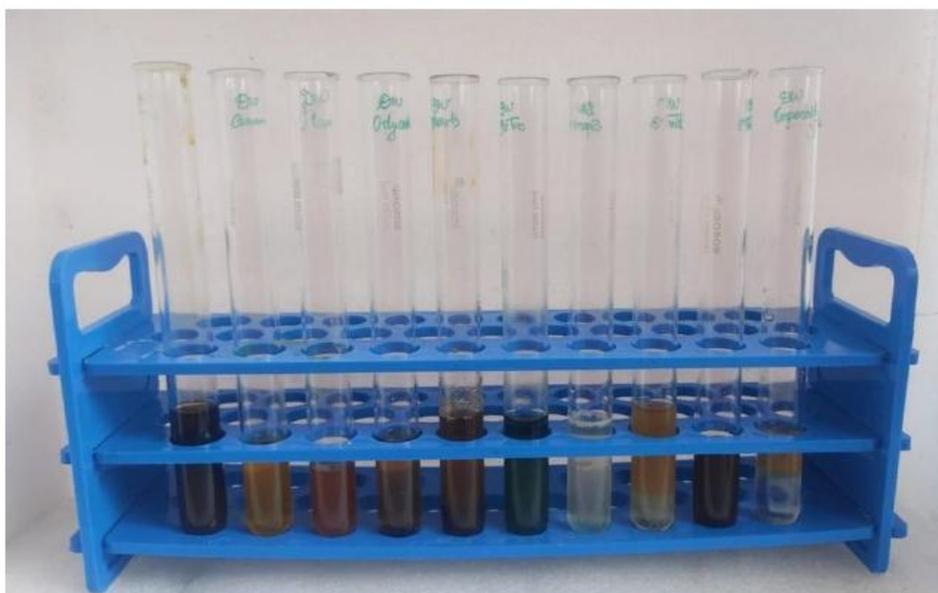
Phytochemical analysis of *scoparia dulcis*



Ethanollic extract of *scoparia dulcis*



Toluene extract of *scoparia dulcis*



Distilled water extract of *scoparia dulcis*

6. DISCUSSION

In the present study, a comprehensive investigation was conducted involving the meticulous isolation and meticulous screening of the uropathogenic bacteria. This highly intricate process aimed to discern the potential effects of diverse extracts derived from the esteemed *S. dulcis* plant species on these bacterial isolates, with the ultimate objective being to ascertain whether these extracts possess any lethal properties against these organisms. As a result of this research work, the findings emerged in a multitude of enlightening and valuable ways, thereby contributing significantly to the existing body of knowledge in this field.

The isolation was done on Hichrome UTI agar other than using the normal uropathogenic organism's isolation agars such as; Cysteine Lactose Electrolyte Deficient

Agar (CLED agar), and Xylose Lysine Deoxycholate (XLD) agar, due to its ability to distinguish most of the uropathogens and their growth. It enables us to use different chromogenic substances for differentiation. It seems to be more unique and the most appropriate agar can be used for the differentiation of uropathogens was proved by Mohamed Khalid (2021) in his work Comparison of Chromogenic (HiCrome Urinary Tract Infection Agar) Medium with Cysteine Lactose Electrolyte Deficient Agar in a Resource-Limited Setting.

The biochemical analysis provided conclusive evidence that the organisms that were isolated possessed the specific characteristics that had already been verified in the agar media that selectively supported their growth. The similar findings was observed by Nabil karah et al.,

2020 in his research activities.

The experimental procedure known as the biofilm screen was performed to assess the capability of bacteria to generate a plaque-like film on the inner walls of the urinary tract. The objective of this endeavor was to determine the propensity of certain organisms to adhere to and proliferate on the aforementioned surfaces. The results of this investigation indicated that *Streptococcus spp.*, and multiple other organisms, specifically *Bacillus* and *Staphylococcus*, exhibited a high degree of biofilm formation. On the other hand, organisms such as; *E. coli*, *Pseudomonas*, and *Enterococcus faecalis* displayed a less pronounced propensity for biofilm formation, demonstrating only moderate levels of adherence. Consequently, it can be inferred that the aforementioned organisms possess the potential to pose a threat to the urinary tract over a prolonged time. The study conducted by Z.Naziret *et al.*, 2021, concludes that the uropathogen *E. coli* shows high biofilm activity over 77% of the isolates were found to be biofilm producers.

The presence of these biofilm formers within the urinary tract can have a detrimental impact on the efficacy of medication administered to patients. The formation of biofilms can impede the absorption and distribution of medication, thereby hindering the therapeutic effects of the drugs. Moreover, the existence of biofilm formers within the urinary tract may foster the emergence of multi-drug resistance among uropathogenic bacteria. This phenomenon occurs when bacteria adapt and develop resistance to multiple types of antimicrobial agents, rendering conventional treatment methods ineffective. As a consequence, the presence of biofilm formers can lead to the development of severe urogenital tract infections and other complex diseases. These infections can cause significant morbidity and can pose a challenge in terms of treatment options, thereby necessitating the exploration of alternative therapeutic strategies. In conclusion, the identification and understanding of the biofilm-forming capabilities of different uropathogens is of utmost importance to develop effective preventive measures and treatment modalities to combat these infections. The study of Maim *et al.*, 2024 evidenced that the multidrug resistance can be mediated by biofilm formation. Biofilm related antimicrobial resistance shown by microbes grown in planktonic culture. Alternative therapies have been sought to remove or inhibit biofilm because antibiotics are not able to treated.

The qualitative analysis of phytochemicals serves as a vital tool in ascertaining the presence and abundance of various compounds within different extracts. The outcome of this analysis revealed intriguing findings. Specifically, the ethanolic extract exhibited positive results in terms of the presence of various essential compounds, including carbohydrates, coumarins, flavonoids, glycosides, phenols, proteins, saponins, steroids, tannins, and terpenoids. Conversely, the toluene extract showcased positive results for the compounds

coumarins, flavonoids, glycosides, proteins, and steroids. Moreover, the distilled water (DW) extract demonstrated positive results for carbohydrates, coumarin, flavonoids, proteins, and saponins. These discoveries shed light on the diverse composition and chemical makeup of the plant extracts, highlighting the presence of a wide range of bioactive compounds that hold significant potential for various applications in the fields of medicine, pharmacology, and natural product chemistry. Similar findings was noted by Ahemed *et al.*, 2022 that the methanolic extract on phytochemical screening gives an insight on the presence of carbohydrate, glycoside, tannins, flavonoids, saponins, triterpenoids and alkaloid in the plant *S.dulcis*.

In the case at hand, this analysis has revealed that the ethanolic extract exhibits a higher number of compounds compared to both the toluene and distilled water extracts. As a result, it can be deduced that the ethanolic extract surpasses the other two compounds in terms of its overall composition. This notable difference can be attributed to the contrasting polarities of the solvents employed in the extraction process.

In the study conducted by Sophy Jose *et al* (2017), the methanolic extract and distilled water extract were used against human pathogens, where it showed the positive result on all the organisms tested. However, in this study, while the antibacterial well diffusion method was conducted, it was observed that all the microorganisms tested were impacted by the ethanolic extract of *S. dulcis*. In contrast, the toluene and distilled water extracts did not exhibit any susceptibility towards the microbes. The formation of zones of inhibition was found to be more pronounced in *Streptococcus*, *Staphylococcus*, and *Pseudomonas*, indicating that the ethanolic extract possesses a high level of inhibitory activity against the growth of these microbes.

The analysis conducted using Gas Chromatography-Mass Spectrometry (GCMS) reveals the existence of a total of 179 chemical compounds, out of which 20 exhibit significant bioactivity. These 20 compounds possess a remarkable range of medicinal properties, including potent anti-microbial, anti-tumor, anti-oxidant, and anti-inflammatory effects. Among these compounds, betulin stands out with its exceptionally high peak value of 8736319. Betulin is a derivative of the terpenoid substance and is exclusively found in the ethanolic extract of *S. dulcis*. In contrast, the toluene and distilled water extracts do not exhibit any presence of terpenoid compounds. The study by Wankapar *et al.*, 2015 proved that the GCMS analysis of the plant *S. dulcis* shows the presence of different phytochemicals in methanol extract namely 2-hexyldecanoic acid methyl ester, methyleicosonite etc. with maximum peak percentage of 51.51%.

Hence, it is reasonable to infer that the formation of an inhibition zone observed in the antimicrobial test

conducted with the ethanolic extract is primarily attributed to the presence of the betulin compound. This significant finding can be thoroughly examined and explored in the context of developing improved drug designs for the treatment of urinary tract infections (UTIs). The results obtained from this study demonstrate the potential efficacy of betulin in combating biofilm producers, thereby indicating its suitability for targeting persistent infections caused by antibiotic-resistant bacteria.

The identification and characterization of these bioactive compounds provide valuable insights into the diverse chemical constituents present within *S. dulcis*. This knowledge can further contribute to the development of innovative therapeutic approaches for various diseases. The high degree of bioactivity exhibited by these compounds underscores their immense pharmacological potential and encourages further investigation into their mechanisms of action. Moreover, the unique properties displayed by betulin highlight its particular significance in the context of anti-microbial and anti-inflammatory interventions.

The compounds possess these functions: Neophytadiene - analgesic, antipyretic, anti-inflammatory, antimicrobial, and antioxidant compound. Hexadecanoic acid, -antioxidants, hypocholesterolemic, nematocidal, and pesticide. 9,12,15-Octadecatrienoic acid -anti-inflammatory. Phytol-antinociceptive and antioxidant activities as well as anti-inflammatory and antiallergic effects. Squalene- as an emollient, antioxidant, and hydrate for the skin. Betulin: Anti-inflammatory, Antiviral, Antibacterial, Antifungal, Anticancer, Anti-HIV, Antimalarial, Antidiabetic, Anthelmintic, and Antioxidant. dl- α -Tocopherol: Antioxidant Campesterol: inhibits the growth of certain bacteria. Stigmasterol: reduces cholesterol levels by limiting the amount of cholesterol that can enter the body. 3-O-Acetyl-6-methoxy-cycloartenol: synthesis of many plant steroids. Lup-20(29)-en-3-ol, acetate,(3 β .):- anti-inflammatory, antioxidant, anticancer, and antimicrobial properties.

Moreover, the employment of Gas Chromatography-Mass Spectrometry (GCMS) as a scientific method for analysis offers a precise means of both identifying and quantifying these substances. This capability enables researchers to delve deeper into the intricate chemical makeup of these compounds, facilitating a more thorough comprehension of their properties. Such a thorough examination plays a crucial role in pinpointing the specific components accountable for the observed biological functions, thereby providing valuable insights for future endeavors in drug discovery. Through the elucidation of the chemical structures and bioactive attributes of natural extracts, these investigations set the stage for the creation of innovative therapeutic agents designed to deliver heightened effectiveness and minimize adverse reactions.

After the DPPH analysis was conducted, the findings indicated that the plant extract exhibited a pronounced efficacy in scavenging free radicals. This signifies a noteworthy antioxidant property present in the plant extract, which could potentially contribute to its therapeutic benefits. Furthermore, the results suggest that the plant extract possesses a significant ability to neutralize harmful free radicals, highlighting its potential use in various applications related to health and wellness. M R Mishra *et al.*, 2013 evidenced that the *S. dulcis* plant can be used as an antioxidant. And Rathosooriya *et al.*, 2005 proved that the aqueous solution of *S. dulcis* possesses remarkable antioxidant property in vitro study conducted.

7. SUMMARY

In the current investigation, an all-encompassing and thorough examination was carried out, which entailed the precise and methodical isolation as well as meticulous screening of the uropathogenic bacteria. The primary purpose of this highly complex and intricate procedure was to discern and ascertain the potential effects of a wide range of extracts derived from the esteemed *S. dulcis* plant species on the aforementioned bacterial isolates. The ultimate objective of this meticulous and carefully planned study was to determine whether these extracts possess any lethal properties against these organisms. Consequently, as a direct result of this meticulously designed study, the findings that emerged were not only numerous but also highly enlightening and valuable, thereby making a significant contribution to the existing body of knowledge in this particular field of research.

The complex process of biofilm development within the urinary tract infection is a multifaceted phenomenon that involves numerous intricate mechanisms and interactions. Furthermore, the gradual aging and degradation of biofilm structures contribute to the gradual decline of cellular lines, leading to further complications and exacerbation of the infection. It is noteworthy to mention that both gram-negative and gram-positive organisms possess the capability to form biofilm within the urinary tract as well as the genital tract, thereby emphasizing the significant impact and widespread occurrence of this phenomenon. Consequently, the formation of biofilm within the urinary tract infection can significantly exacerbate the severity and complexity of the infection, further deteriorating the overall health condition of the patient.

The identification of vital constituents, including carbohydrates, coumarins, flavonoids, glycosides, phenols, proteins, saponins, steroids, tannins, and terpenoids, was achieved through an examination of the plant material. Consequently, to comprehensively investigate this initial evaluation, a gas chromatography-mass spectrometry (GCMS) analysis was conducted on the leaf of the aforementioned plant.

The GCMS analysis provides the accurate compounds

present in the sample. In conclusion, the GCMS analysis of the chemical composition of *S. dulcis* extracts has unveiled a plethora of bioactive compounds, including betulin, with remarkable anti- microbial, anti- tumor, anti-oxidant, and anti-inflammatory properties. The presence of betulin in the ethanolic extract suggests its potential role in the observed antimicrobial effects. These findings provide valuable insights for the design and development of novel drugs, particularly in the context of urinary tract infections. Furthermore, the identification and characterization of these compounds contribute to our understanding of their pharmacological potential and pave the way for future research in the field of natural product-based drug discovery.

The study concludes that the leaf of the plant has therapeutic potential in the treatment of Urinary tract infections. The ethanol extract of the *S. dulcis* leaf exhibits positive effects on both gram-positive and gram-negative bacteria. Furthermore, the bacterial isolates demonstrate biofilm properties. Consequently, the study suggests that future research should focus on this plant to obtain more accurate results and develop a novel drug for clinical therapy.

The GCMS analysis reveals that the majority of the compounds possess anti- tumor properties, indicating that the plant-based study can have broad applications in cancer research. Specifically, it may prove beneficial in the study of cervical and ovarian cancers, where further investigation can be conducted. Additionally, the procedures for drug design, as well as in vitro and clinical trials, can transform this plant product into a valuable therapeutic agent. The antioxidant property displayed by a substance indicates a significant therapeutic value, making it a crucial element for various treatments. The study of cancer in plants is highly important due to its valuable role in eliminating free radicals, which are detrimental to the body. Moreover, the compounds found in GC also support the validity of this assertion.

8. CONCLUSION

The significance of treating pathogens with natural products is highlighted in the study. *Scoparia dulcis*, a plant known for its traditional use in managing urinary tract and kidney issues as well as diabetes, exhibits a potent antibacterial effect through the lethal activity of its leaf extract. The findings of this research suggest a promising avenue for drug development and clinical trials involving betulin, a compound believed to originate from this plant, which could potentially serve as a source for inhibiting the uropathogens that have been identified. Therefore, this investigation serves as a valuable resource for further exploration into isolating and purifying the compounds found in the aforementioned leaf extract, paving the way for an in-depth examination aimed at transforming it into a viable pharmaceutical product.

Moreover, the antioxidant properties demonstrated by the plant extract (Aqueous) underscore its ability to combat free radicals, a crucial aspect highlighted in the study. This attribute not only emphasizes the importance of eliminating harmful radicals but also indicates a secondary benefit of the research. The insights gained from this study could potentially effective advancements in cancer treatment, with the plant extract showing promise in this area as well. Consequently, the application of these findings in the realm of drug development holds significant potential for enhancing therapeutic approaches in the field of oncology.

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