Indian Journal of Biochemistry & Biophysics
Vol. 61, March 2024, pp. 182-189
DOI: 10.56042/ijbb.v61i3.8225

In vitro efficacy of phytotherapeutics for the prevention of urinary tract infections in pregnant women

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Received 22 January 2024; revised 10 February 2024

Urinary tract infections (UTIs) during pregnancy pose significant challenges due to limited treatment options to avoid potential harm to the fetus. This study investigates the *in vitro* efficacy of phytotherapeutics, specifically *Ocimum sanctum* L. (Tulsi), in preventing urinary tract infections in pregnant women. UTIs, a prevalent hospital-acquired infection and a leading cause of bacteremia in hospitalized patients, contribute to adverse pregnancy outcomes if left untreated. The research explores the potential of Tulsi, known for its diverse therapeutic benefits, including antimicrobial properties. Traditional uses as an expectorant, analgesic, anti-asthmatic, and anti-diabetic agent underscore its holistic potential in combating infections. Previous studies suggest that *Ocimum sanctum* L.-containing herbal medicines may mitigate the duration of sickness, clinical symptoms, and biochemical markers associated with bacterial and viral infections. This investigation aims to assess the *in vitro* efficacy of *Ocimum sanctum* leaf extract, offering valuable insights into its preventive potential against UTIs in pregnant women. The findings may contribute to the development of safe and effective phytotherapeutic interventions for managing UTIs during pregnancy.

Keywords: Antibacterial activity, *Ocimum sanctum*, Plant based drug, Urinary tract infections

A medicinal plant is defined as an herbal preparation created from plant materials through processes such as fractionation, extraction, concentration, purification, or other biological or physical methods. These preparations can be crafted for immediate consumption or serve as a basis for herbal products, as outlined by the World Health Organization (WHO) in 2003. Given their diverse array of antibacterial chemicals, medicinal plants play a crucial role in the treatment of bacterial illnesses[2].The traditional use of medicinal plants for remedies is deeply. In grained in the rural regions of many developing nations, as acknowledged by Sandhu and Henrich[35]. This practice has stood the test of time, emphasizing the cultural and historical significance of medicinal plants in healthcare. Notably, medicinal plants have the potential to produce compounds that are inherently toxic to microbes yet safe for human consumption, rendering them cost-effective and sustainable sources of pharmacologically active molecules, as demonstrated by Basile *et al*[7].

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Researchers are exploring natural bioactive chemicals derived from medicinal plants as an alternative to conventional antibiotics and synthetic antimicrobial agents[20]. These bioactive chemicals encompass various classes of organic compounds, including nucleic acid bases, lipids, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), terpenoids, peptides, amino acids, alkaloids, steroids, proteins, carbohydrates, saponins, flavonoids, and phenol compounds[30,44]. The World Health Organization (WHO) estimates that more than 80% of people worldwide, especially in developing nations, rely on plant extracts and their active compounds as part of traditional folk medicine integrated into conventional pharmaceutical practices[19]. An increasingly critical global health concern is the escalating resistance of bacteria, viruses, and fungi to antibiotics, coupled with the limited accessibility of certain medications commonly employed for treating infectious disorders[34]. In contemporary times, natural materials are gaining prominence as valuable reservoirs of novel drugs. Surprisingly, over half of the pharmaceuticals administered in clinical settings continue to be composed of natural compounds or their derivatives, underscoring the enduring significance of these

resources[11]. A urinary tract infection (UTI) refers to an infection in the urinary tract, and it stands as one of the most prevalent ailments globally, especially affecting women[27]. Bacteria represent the predominant cause of UTIs. Typically, the body swiftly eliminates germs entering the urinary tract before they manifest symptoms (World Health Organization, 2005). Nevertheless, infections may occasionally occur when bacteria manage to bypass the body's defense mechanisms.

UTIs are primarily caused by the enterobacteriaceae family, including bacteria such as *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), and others[3]. Morad Asaad *et al*[28] highlight that the situation has been exacerbated by the emergence of multi-drug resistant pathogenic bacteria like *E. coli, K. pneumoniae, and Staphylococcus saprophyticus* (*S. saprophyticus*), responsible for UTIs and other ailments. Consequently, there is a pressing need to replace existing medications with new antibacterial substances that are effective against bacteria resistant to multiple treatments while remaining safe for human use[37].

Urinary tract infections are prevalent among pregnant women, presenting a noteworthy challenge in their treatment[14]. The conventional course of action, involving antibiotics, is hindered during pregnancy due to potential risks to the fetus, including adverse outcomes such as death or birth defects[1].This underscores the imperative for alternative approaches and medications to address UTIs in pregnant women without compromising the health of both the mother and the unborn child. Microbial infections are a frequent cause of human urinary tract infections (UTIs) and nosocomial diseases. In underdeveloped nations, human UTIs account for nearly 35% of hospitalized patients, making them a widespread health concern[13]. The prevalence is higher in sexually active women and further elevated in individuals with diabetes, sickle cell disease, and anatomical urinary tract malformations. Men, particularly those with enlarged prostate glands, and patients with indwelling bladder catheters, are also at an increased risk of bacteriuria and UTIs[24].

Urinary tract infections (UTIs) have become the leading hospital-acquired infection and the second most common cause of bacteremia in hospitalized patients, constituting up to 35% of nosocomial infections[39]. If left untreated, UTIs can lead to severe complications such as intrauterine growth restriction, preeclampsia, premature births, and the necessity for cesarean sections[26]. *Ocimum sanctum* L., commonly known as the Tulsi plant, offers a myriad of therapeutic benefits. Traditional medical practitioners have utilized this plant as an expectorant, analgesic, antiasthmatic, anticancer, antimicrobial, antifertility, antidiabetic, hypolipidemic, hypotensive, and antistress agent. Different parts of the plant, including leaves, flowers, stem, root, and seeds, are recognized for their therapeutic potential. Studies suggest that herbal medicines containing *Ocimum sanctum* L. can potentially reduce the duration of sickness, clinical symptoms, and biochemical markers in patients with bacterial and viral infections[31,43]. The objective of this investigation is to assess the *in vitro* efficacy of *Ocimum sanctum* leaf extract against urinary tract infections in pregnant women.

Materials and Methods

Collection of *Ocimum sanctum*

The leaves of *Ocimum sanctum* were procured from an herbal market in Watrap, Virudhunagar district. Upon collection, these leaves underwent a meticulous washing procedure with sterile water to remove any dust and foreign particles. Following the cleansing step, the leaves were carefully dried in a dark room. Once completely dehydrated, the leaves were finely ground into a powder to enhance the efficiency of the extraction process.

Preparation of plant extract

Methanolic extraction

A quantity of 250 mg of *Ocimum sanctum* leaf powder was combined with 10 mL of 70% methanol and subjected to overnight shaking. The resulting suspension was then filtered through filter paper and transferred to a Petri dish for the purpose of methanol evaporation, ultimately yielding a powdered form[15].

Aqueous extraction

Furthermore, 300 g of air-dried *Ocimum sanctum* leaf powder underwent a meticulous blending process with 600 mL of sterile distilled water. The resultant solutions were allowed to stand at room temperature for a 3-day maceration period, during which they were gently swirled to ensure thorough mixing. Following this maceration period, the mixtures underwent filtration through muslin cloth to eliminate any solid particulate matter. Subsequently, the filtrate underwent centrifugation at 5000 revolutions per minute for a duration of 15 min. The resulting supernatant underwent an additional filtration step using Whatman Filter No. 1 paper. The final extract

was obtained through the evaporation of the liquid, leaving behind the desired extract in a dry state[6].

Phytochemical analysis

A qualitative phytochemical screening was conducted on each *Ocimum sanctum* extract to confirm the presence of bioactive compounds.

Flavonoid test (Ammonia test)

For the assessment of flavonoids using the Ammonia Test, a 5 mL portion of dilute ammonia was combined with 1 mL of the extract. Subsequently, 1 mL of concentrated HCl was added to the solution. The appearance of a yellow color in the solution serves as an indicator of the presence of flavonoids[34].

Alkaloid presence test (Wagner's test)

To determine the presence of alkaloids using Wagner's test, 0.5 g of the extract was treated with 1 mL of dilute hydrochloric acid (HCl). Another test tube containing 1 mL of the extract was utilized, and 1 mL of potassium iodide (Wagner's reagent) was added and shaken. The formation of a reddish-brown precipitate confirmed the presence of alkaloids[10].

Tannin test

In the tannin test, 0.5 mL of the extract was diluted with 1 mL of distilled H2O. Following this, 2-3 drops of 0.1% ferric chloride were introduced to the solution. The development of a brownish-green or blue-black coloration in the solution confirmed the presence of tannins in the extract[9].

Saponin test

To assess the presence of saponins, 1 mL of the plant extract was added to 5 mL of distilled water, and the mixture was vigorously shaken. The formation of a stable foam lasting up to 40 min indicated the presence of saponins[34].

Phenol test (Ferric chloride test)

To conduct the Ferric chloride test for phenol, 2 mL of alcohol and 2-3 drops of ferric chloride solution were introduced to 1 mL of the extract. The development of a bluish-green or black coloration signifies the presence of phenolic compounds[6].

Glycosides test

For the detection of glycosides, 1 mL of acetic acid and 2 drops of ferric chloride were combined with 2 mL of the extract. Subsequently, 2 mL of concentrated H_2SO_4 was added, resulting in the formation of a reddish-brown coloration, indicating the presence of cardiac glycosides[34].

Steroid test (Libermann burchard's test)

In the assessment of steroids using Libermann Burchard's test, an alcoholic extract is first evaporated to dryness and then re-extracted with chloroform. A few drops of acetic anhydride are added, followed by sulfuric acid along the side of the test tube.

The appearance of a violet to blue-colored ring at the junction of the two liquids suggests the presence of steroids[34].

Diterpenes test (Copper acetate test)

For the detection of diterpenes, 1 mL of the extract is treated with 3-4 drops of 1% copper acetate solution for 3 seconds. The formation of an emerald green coloration indicates the presence of diterpene[33].

Carbohydrate test (Molisch's test)

In the Molisch's test for carbohydrates, 2 mL of Molisch's reagent is added to 0.5 mL of crude extract, and the mixture is thoroughly shaken. Following this, 2 mL of concentrated H2SO4 is carefully poured along the side of the test tube. The appearance of a violet ring at the interface indicates the presence of carbohydrates[8].

Protein test (Xanthoproteic test)

To perform the Xanthoproteic test for proteins, 1 mL of the extract is placed in a test tube, and a few drops of nitric acid are added and shaken. The emergence of a yellow color indicates the presence of proteins[1].

Antioxidant activity of plant extract

The assessment of scavenging activity in plant extracts was conducted through the DPPH assay. DPPH solutions were meticulously prepared in methanol. Subsequently, stock solutions (1 mg/mL) of the plant extract and the standard ascorbic acid (0.05 g/mL) were prepared using methanol. A range of concentrations for both the plant extract and ascorbic acid (200 to 800 mg/mL) was examined in this study[16].

To measure the scavenging activity, absorbance readings were taken at 520 nm. The radical scavenging activity was determined using the following formula:

$$\text{Radical Scavenging Activity (\%)} = [1-(\text{Absorbance of sample}/\text{Absorbance of control})] \times 100$$

This formula allows for the calculation of the percentage of radical scavenging activity based on the

absorbance readings, providing valuable insights into the antioxidant potential of the plant extracts[16].

Collection and culturing of urine samples

Therefore, a total of 10 urine samples were gathered from the Government Hospital in Sevalpatti, Virudhunagar district. These samples were acquired from individuals aged between 25 to 35 years who were diagnosed with urinary tract infections. The collected urine samples underwent streaking onto sterile EMB (Eosin Methylene Blue) agar medium. The prepared medium was poured into sterile Petri plates and left to solidify at room temperature. Following solidification, the urine samples were streaked onto various plates. Subsequently, the plates were incubated aerobically at 35-37°C for 18-24 h, shielded from light. The examination of the plates was then conducted based on colonial morphology.

Identification of UTI pathogens

Colonies obtained from the cultured plates were chosen and characterized using morphological, cultural, physiological, and various standard biochemical methods. Identification of different organisms from the cultured plates was based on the examination of colony color and morphology.

Antibacterial screening

The antibacterial activity of plant extracts against clinical isolates of urinary tract infections (UTI) was assessed using the agar well diffusion method[34]. Mueller Hinton agar served as the medium for antibacterial testing. Broth cultures of the test bacteria were aseptically swabbed onto sterile Mueller Hinton agar plates using cotton swabs. Two wells, each with a diameter of 5mm, were created on the surface of the Mueller Hinton agar using a sterile cork borer. Aqueous and methanol extracts at various concentrations were added to the labeled wells. The inoculated plates were then incubated at 37°C for 24 h, and the zone of inhibition was recorded.

Statistical analysis

The experiment was performed in triplicates. The results are represented as Mean ± Standard deviation (SD).

Results

Phytochemical analysis

Phytochemical analysis was conducted on extracts of *Ocimum sanctum*, revealing the presence of various compounds such as flavonoid, alkaloid, cardiac glycoside, diterpenes, steroid, tannins, phenol, saponin, carbohydrate, and protein. The interpretation of results was based on the intensity of the observed color, and qualifications were assigned as Highly present (+++), Moderately present (++), Slightly present (+), and Absent (-) in the methanolic extract of *Ocimum sanctum.*(Table 1).

Antioxidant activity of plant extract

The extract from *Ocimum sanctum* exhibits robust antioxidant activity against all the tested free radicals. Ascorbic acid serves as the control, with methanol retained as the blank. The DPPH radical, commonly utilized to assess free radical scavenging activity, exhibited an 82% reduction in methanol at a concentration of 800 µg/mL in the leaf extracts. (Table 2 & Fig. 1).

Antibacterial screening

On investigation, the organism was grown on EMB Agar as green metallic sheen so based on the colour

Table 1 — Phytochemical analysis of *Ocimum sanctum* extracts

Phytochemicals constituents	Observation
Flavonoid	++
Alkaloid	+
Cardiacglycoside	+++
Diterpenes	++
Steroid	-
Tannins	+
Phenol	++
Saponins	+++
Carbohydrate	+
Protein	+

Table 2 — Antioxidant activity of Tulsi extract

Concentration (mg/mL)	OD at 520 nm	% inhibition
200	0.390	62%
400	0.323	68%
600	0.221	78%
800	0.180	82%

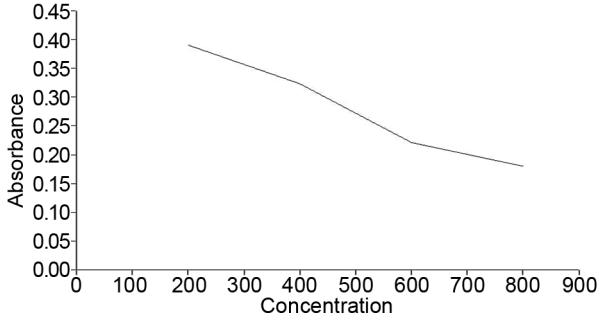

Fig. 1 — Graph for Antioxidant activity

Table 3 — Antibacterial activity of *Ocimum sanctum* extracts against *E. coli*

Samples	Concentration (µg/µL)	Zone of inhibition (mm)
Aqueous extract	50	10 ± 0.1
	100	12 ± 0.1
Methanolic extract	50	16 ± 0.2
	100	20 ± 0.3
Streptomycin (positive control)	50	17 ± 0.2
	100	22 ± 0.4

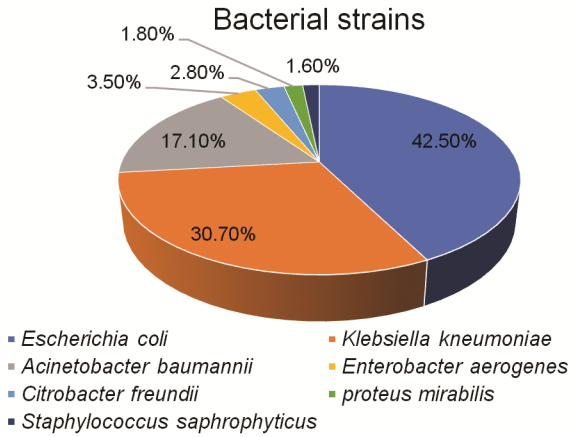

Fig. 2 — Bacterial strain screening

and morphology of colonies, we identified the *E. coli* which are mostly present in the urine samples. Table 3 shows the antibacterial activity of *Ocimum sanctum* extracts against *E. coli.*(Fig. 2).

In this study, bacterial strains employed were derived from both uncomplicated and complicated urinary tract infections (UTIs). The array of strains encompassed *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterobacteraero genes, Acinetobacter baumannii, Citrobacter freundii,* and *Staphylococcus saphrophyticus.*

Discussion

The phytochemical screening of *Ocimum sanctum* leaf extracts, as conducted by Barnabas *et al*. (2022)[6], revealed the presence of substances known for their physiological and biological activities. Specifically, the methanolic extracts of *O. sanctum* exhibited the presence of flavonoids, alkaloids, cardiac glycosides, diterpenes, steroids, tannins, phenols, saponins, carbohydrates, and proteins, as identified through phytochemical assays. This aligns with the findings of Borah *et al*. (2018)[8,33], who emphasized the presence of phytochemicals in plant extracts. Notably, the investigation highlighted the substantial concentration of flavonoids, cardiac glycosides, phenols, saponins, and diterpenes in *O. sanctum*. This finding is consistent with Borah *et al* (2018)[8] discovery of high concentrations of flavonoids, saponins, cardiac glycosides, and phenols in *Ocimum sanctum* leaves, attributing these phytochemicals to the leaves' antibacterial properties. Phenolic compounds, particularly polyphenols and flavonoids, constitute a major class of plant metabolites with various biological qualities, as outlined by Mann (2012)[25], including anti-apoptosis, cardiovascular protection, anti-inflammation, anti-atherosclerosis, anti-aging, anti-carcinogenesis, improved endothelial function, and inhibition of cell proliferation. Flavonoids, recognized as hydroxylated phenolic compounds, are produced by plants in response to microbial infection. The research by Barnabas *et al*. (2022)[6] affirmed the antibacterial properties of flavonoids against diverse pathogens *in vitro*. This antimicrobial activity is likely driven by their ability to form complexes with bacterial cell walls and extracellular soluble proteins. Additionally, alkaloids, naturally occurring nitrogen-containing chemicals, have been reported to possess antimicrobial effects due to their ability to intercalate with the DNA of microbes[42,45].

According to Telci *et al*. (2006)[40,47], phenolic chemicals such as flavonoids and tannins are likely responsible for the free radical scavenging action. Additionally, flavonoids exhibit a range of medicinal properties, including antibacterial, anti-allergic, antiviral, anti-platelet, anticancer, hypoglycemic, antioxidant, and anti-inflammatory effects[18,46]. Tannins, known for their ability to deactivate and eliminate disease-causing microbes, also play a crucial role in these biological activities[32]. Terpenes, including diterpenes, have been observed to exert various biological actions in animals and microbes, such as antibacterial, anti-inflammatory, and hormonal effects. Certain tannins have documented diuretic and antiviral properties, while saponins and cardiac glycosides serve as both cardio tonics and antifungals. Steroidal compounds have also been reported to possess antibacterial and antidiabetic qualities[5].

The antioxidant activity of the methanolic extract of Tulsi was determined through the DPPH method, where the absorbance decreases due to a color change from dark green to yellow. This assay, based on the reduction of the stable DPPH radical in the presence of a hydrogen-donating antioxidant, is commonly used to screen antioxidant activity of natural compounds. Results indicated that the methanolic

extract of Tulsi exhibited significant antioxidant capacities compared to vitamin C as a standard antioxidant compound.However, the aqueous extracts of *O. sanctum* displayed only mediocre antibacterial properties in the current study. This may be attributed to the polarity of water as an extraction solvent, potentially missing the extraction of non-polar compounds. The higher yield of the aqueous extract compared to the methanolic extract suggests that a considerable number of bioactive compounds may not have been extracted by water. This contrasts with the suggestion by Lapornik *et al*. (2005)[21, 48] that water is a superior extraction solvent compared to methanol. The findings align with Das *et al*. (2010)[12], who emphasized that water-soluble phenolics are significant as antioxidant compounds, while water-soluble flavonoids have limited antibacterial value. Despite the common use of water by traditional healers for extraction, plant extracts from organic solvents have demonstrated more consistent antibacterial activity, as observed in this study. The antibacterial activity of the plant extracts against *E. Coli* varied with concentration in the current investigation.

The methanolic extracts exhibited potent activity against UTI pathogens at various doses, with the zone of inhibition increasing at higher concentrations and decreasing at lower concentrations[6]. The observed high activity of methanolic extracts may be attributed to the polarity of the chemicals in the leaves or the extraction solvent (methanol), given its polar nature. The enhanced activity of methanolic extracts compared to aqueous extracts is likely due to the higher levels of polyphenols present. This aligns with research suggesting that methanol is among the most effective solvents for extracting fresh *O. sanctum* leaves[8]. Methanol's efficiency in extracting plant active components important for medicinal purposes has been acknowledged, with studies emphasizing its superior diffusion into the medium compared to water, both cold and hot[29].Moreover, methanol's ability to easily pass through cellular membranes to collect intracellular components from plant material has been established[41]. Initial methanol extraction is commonly employed to obtain known active components from plants, particularly aromatic or saturated organic compounds with antimicrobial properties. Given the substantial concentrations of phenols, flavonoids, cardiac glycosides, and saponins in *Ocimum sanctum* leaf extracts, their potent antibacterial activity against both Gram-positive and Gram-negative pathogens is evident. Numerous studies, including[34], have highlighted *Ocimum sanctum's* antibacterial action against fungi and bacteria. It is important to note that the bioactive components extracted from *Ocimum sanctum* leaves using methanol were responsible for inhibiting uropathogens, not the methanol itself. Positive controls, such as streptomycin, also demonstrated suppression of uropathogens at different concentrations. The similarity in the zones of inhibition between plant extracts and antibiotics suggests that both possess antimicrobial properties, emphasizing the potential of plant extracts as effective alternatives in combating microbial infections.

Conclusion

The exploration of plant-based alternatives to antibiotics for preventing and treating urinary tract infections (UTIs) is gaining momentum. These alternatives offer advantages such as cost-effectiveness, ready availability, safety, reduced side effects, lower risks of antimicrobial resistance, and the potential to mitigate adverse antibiotic effects. In our *in vitro* experiments, specific phytotherapeutic agents have demonstrated the ability to inhibit the growth of uropathogenic bacteria commonly associated with UTIs in pregnant women. The research and experimentation conducted in this study have yielded promising results, particularly in the efficacy of a Tulsi-based ointment for treating UTIs. This suggests that Tulsi, with its antimicrobial and anti-inflammatory properties, could be a valuable component in developing UTI treatments. However, it is crucial to emphasize the need for further research to validate these findings through clinical trials and to assess the safety and effectiveness of the ointment when applied to UTI patients.

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