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## Comparative Study on Antibacterial Potential of Phytochemicals and Ayurvedic Formulations against Urinary Tract Infectious Bacteria

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### **Abstract:**

Medicinal plants and Ayurvedic formulations are an abode of suitable plant extracts identified for their active compounds and are devised against suitable infections. This natural treatment is widely accepted for their efficacy, safety and lesser side effects. The present study involves identifying the most suitable antibacterial agent among phytochemicals and ayurvedic formulations for the cure of urinary tract infections which is a highly prevalent disease in the present scenario. *E. coli*, *Klebsiellapneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* identified from urine samples were used as test strains. The disc diffusion assay was performed for a comparative analysis to identify the most suitable plant extract, its active compound and Ayurvedic formulation, that could be used in curing ailments of Urinary Tract Infections. The plant extracts of *Eupatorium odoratum* L., *Azadirachta indica* L., *Ocimum sanctum* L. *Coleus aromaticus* L., *Aloe barbadensis* L., and *Rotulaaquatica* L. were tested for their antibacterial activity against the isolates. Further, the active compounds of lignins and proteins were isolated from the effective extracts of *Eupatorium odoratum* L. and *Rotulaaquatica* L. and tested for their antibacterial activity which could be used in the formulation of various medicines. A comparative study was also made with the Ayurvedic formulations like *Brihatyadikashayam*, *Viratradikashayam*, *Chandraprabhavatika* and *Punarnavasavam*, to identify its antibacterial efficacy. The most effective plant extracts were that of *Eupatorium odoratum* L. and *Rotulaaquatica* L. The protein extract of *Eupatorium odoratum* L. and *Rotulaaquatica* L. proved to be effective constituent as antibacterial agent. Among the Ayurvedic formulations, *Chandraprabhavatika* exhibited bacteriostatic activity against *Klebsiellapneumoniae* and *Staphylococcus aureus*. The excessive use of antibiotics paves way for secondary ailments and resistance between different bacterial species, hence there is a rapid replacement of chemical antibiotics with natural remedies which is highly demanded for their non toxicity and lesser side effects and better cure.

**Keywords:** Phytochemicals, Ayurvedic formulations, Antibacterial, Active compounds

### **1. Introduction**

Medicinal plants are nature's hidden extend of unexplored treasure which are replacing the chemical antibiotics in the pharmaceutical industry. Medicinal plants are highly accepted now-a-days for their efficacy and safety which finds a greater application in Ayurvedic formulations. (Ravikumar *et al.*, 2010)

One of the highly precedented diseases of present scenario is urinary tract infections incident mainly due to lack of drinking pure water as well as finding a substitute for water. Four strains of bacteria, *Escherichia coli*, *Klebsiellapneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are highly prevalent in catheterized urinary sample (Raj and Joseph, 2016). Today large number of population suffers from kidney stones, gallstones and urinary calculi and are switching over to natural cure because of their promising efficacy and safety.

Medicinal plants are frequently used as raw materials for extraction of active ingredients which is used in the synthesis of different drugs. (Sharma *et al.*, 2009). Curing ailments by natural cure is highly effective because the ingredients of plants all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects (Sensiet *et al.*, 2003).

Although modern antibiotics are being used in UTIs, urinary tract infections can be quickly and easily treated with herbal treatments with no side effects. Herbs known for the management of urinary tract infections and other urinary disorders are divided into important categories like Urinary antiseptic and anti-adhesion herbs, bladder protectives that control bladder from infections, kidney care and herbs for symptoms of benign prostatic hyperplasia. These herbs are discerned to possess different type of phytoconstituents and exhibit potential in the treatment of urinary disorders and could be an alternative to uropathogen resistance to the antibiotic during a UTI (Adgegoke *et al.*, 2011).

Urinary tract infections can also be correlated to Mutrakrichra in Ayurveda. Mutrakrichra can be due to Vata,Pitta or Kapha. Ayurveda has special formulations to manage mutrakrichra. These medicines help to alleviate the inflammation, dissolve the urinary calculus to a certain extent and protect urinary system including kidneys. (Kumar *et al.*, 2012).

The present study was undertaken to analyze the comparative effect of phytochemicals and ayurvedic formulations against bacteria present in urine samples and their susceptibility patterns to selective plant extracts and hence identifying the efficacy of the formulation as well as the most effective antibacterial agent eradicating the disease.

## 2. Materials and Methods

The urine sample was obtained by catheterization and identified by preliminary investigation as *Escherichia coli*, *Klebsiellapneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and confirmed using biochemical analysis. Further a comparative study was carried out to study the effect of phytochemicals and ayurvedic formulations against the bacterial strains.

### 2.1. Selection of Plants

The antibacterial analysis of plant extracts was done using plant extracts, reported to have potential for curing Urinary Tract Infections and is a chief ingredient of ayurvedic formulations. The plants having potential for curing urinary tract infections that were used and the parts that were selected for the study included the leaves of *Azadirachtaindica*L., *Eupatorium odoratum*L. *Ocimum sanctum* L., *Coleus aromaticus*L., and *Aloe barbadensis*L.and the stem of *Rotulaaquatica*L.

#### 2.1.1. Preparation of the Plant Extracts

The plant extracts were prepared by washing the plant parts with distilled water 2-3 times for removing the dirt and other soil contaminants, and 20gm of the plant part was accurately weighed and ground to a paste with 50 ml of 100% acetone.

10 concentrations of the plant extract, from 100% to 10%, were selected for disc diffusion plating method.

The plant extracts which proved ideal were further subjected to isolation of lignins and proteins which were tested for antibacterial action by disc diffusion method. (Kirby-Bauer,1950).

### 2.2. Isolation of Proteins from Plant Tissue

Isolation of Proteins from plant tissue was done by Protein acetylation (Sensiet *et al.*, 2003).

### 2.3. Isolation of Lignins from Plant Tissue

Isolation of Lignin was done by Lignin acetylation (Doherty *et al.*, 2010).

### 2.4. Testing of isolated Proteins and Lignins for Antibacterial Activity

The isolated proteins and lignins were tested for antibacterial activity using disc diffusion method. The plant extracts of *Eupatorium odoratum* L. and *Rotulaaquatica*L. were used for the purpose. The bacterial strains that exhibited the highest inhibition zone were used to test for the effect of proteins and lignins. The petriplates containing the Mueller Hinton agar medium was swabbed with the corresponding bacterial strain and was seeded with the paper discs. Using sterile micropipette 0.5ml of the protein and lignin extracts were introduced onto the paper discs. The petriplates were incubated at 37°C for 24 hours. The clear zone was observed and measured using a scale.

### 2.5. Antibacterial analysis using ayurvedicFormulations

The antibacterial analysis for ayurvedic preparations was done by disc diffusion method using commercially available ayurvedic formulations like Brihatyadikashayam, Chandraprabhavatika, Punarnavasavam, and ViratradiKashayam.

The culture plates were swabbed with the corresponding bacterial strains. Four discs were placed using sterile forceps equally leaving adequate space from the margin. Using a sterile micropipette 0.5ml each of the ayurvedic preparations were added on to the discs. The culture plates were incubated overnight at 37°C for 24hrs. The clear zone observed was measured using a scale.

## 3. Results and Observation

### 3.1. Results

Four different bacterial strains were isolated from catheterized sample and identified using preliminary investigation on the basis of colony characteristics and confirmed using biochemical analysis as *Escherichia coli*, *Klebsiellapneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

#### 3.1.1. Antibiotic Sensitivity Test Using Plant Extracts for Curing Urinary Tract Infections

Among the selected plant extracts, the leaf extracts of *Eupatorium odoratum* L. and stem extracts of *Rotulaaquatica* L. proved to be effective against all bacterial strains at least in one concentration as evidenced from the zone of inhibition exhibited (Table 1).

| PLANT EXTRACTS             | 20%                     |    | 20%                         |    | 20%                           |    | 20%                          |    |
|----------------------------|-------------------------|----|-----------------------------|----|-------------------------------|----|------------------------------|----|
|                            | 80%                     |    | 80%                         |    | 80%                           |    | 80%                          |    |
|                            | <i>Escherichia coli</i> |    | <i>Klebsiellapneumoniae</i> |    | <i>Pseudomonas aeruginosa</i> |    | <i>Staphylococcus aureus</i> |    |
|                            | Zone size in mm         |    |                             |    |                               |    |                              |    |
| <i>Aloe barbadensis</i>    | -                       | -  | -                           | -  | -                             | -  | -                            | -  |
| <i>Azadirachta indica</i>  | 23                      | 15 | -                           | 14 | 14                            | 12 | -                            | -  |
| <i>Coleus aromaticus</i>   | -                       | -  | -                           | 12 | -                             | -  | -                            | -  |
| <i>Eupatorium odoratum</i> | 23                      | 16 | 25                          | 17 | -                             | 17 | -                            | 10 |
| <i>Ocimum sanctum</i>      | 23                      | 13 | 12                          | 10 | -                             | -  | -                            | -  |
| <i>Rotula aquatica</i>     | 20                      | 17 | 16                          | 15 | 21                            | 22 | 12                           | -  |

Table 1: Antibiotic Sensitivity test using plant extracts having potential for curing urinary tract infections

### 3.1.2. Antibacterial Effect of Proteins And Lignins Isolated From Plant Extracts

The protein extracts of *Eupatorium odoratum*L. and *Rotula aquatica*L. were effective against *E. coli* strain exhibiting maximum inhibition zone when compared to lignin extracts (Table :2).

| BACTERIAL STRAINS             | <i>Eupatorium odoratum</i> Protein extracts |           | <i>Eupatorium odoratum</i> Lignin extracts |           | <i>Rotula aquatica</i> Protein extracts |           | <i>Rotula aquatica</i> Lignin extracts |           |
|-------------------------------|---|-----------|--|-----------|---|-----------|--|-----------|
|                               | Zone size in mm                             |           |  |           |   |           |  |           |
| <i>Escherichia coli</i>       | <b>18</b>                                   | 12        | 13   | 10        | 17                                      | 14        | 15                                     | 10        |
| <i>Klebsiella pneumoniae</i>  | 13  | 10        | 12   | 10        | 12                                      | 10        | 13                                     | 10        |
| <i>Pseudomonas aeruginosa</i> | 17  | 15        | 11   | 10        | 13                                      | 11        | 12                                     | 10        |
| <i>Staphylococcus aureus</i>  | Resistant                                   | Resistant | <b>10</b>                                  | Resistant | Resistant                               | Resistant | Resistant                              | Resistant |

Table 2: Antibacterial effect of proteins and lignins isolated from plant extracts using disc plate method

### 3.1.3. Antibiotic Sensitivity Analysis Using Ayurvedic Formulations

Ayurvedic formulations of Punarnavasavam, Chandraprabhavatika and Viratradikashayam were bacteriostatic against *Klebsiella pneumoniae* and Chandraprabha vatika exhibited bacteriostatic activity against *Staphylococcus aureus*

## 4. Discussion

Medicinal plants have been known since millennia for their efficacy and safety and is widely replacing chemical antibiotics which during the course of treatment become resistant to infections (Adgegoke *et al.*,2011). In the present scenario urinary tract infections are highly prevalent due to the lack of drinking pure water and also for finding a substitute for clean drinking water. The infections may arise as a result of catheterization or as a secondary infection. In the present scenario where urinary tract infections are on the

rise, resulting in complex ailments, the plant extracts serve as an ideal antibacterial agent. The urine samples obtained indicated the presence of four different bacterial strains of *Escherichia coli*, *Klebsiellapneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Raj and Joseph, 2016), which were subjected to disc diffusion method of antibacterial analysis using plant extracts and Ayurvedic formulations so as to identify the most suitable antibacterial agent.

Plant extracts have been widely used as antibacterial agents and is found to be the chief ingredient in production of various medicinal formulations in the present scenario, especially for urinary tract infections. In the present study the plant extracts of *Eupatorium odoratum* L. and *Rotulaaquatica* L. were found to be very effective against all bacterial strains. The study on the antibacterial effect of *Eupatorium adenophorum* has proved to be effective against *Proteus* spp., *Salmonella* spp., *Staphylococcus* spp., *Bacillus subtilis*, *B. thurengiensis*, *B. cereus*, *Enterobacter aerogenes*, *Salmonella paratyphi*, *Staphylococcus aureus* and *Proteus mirabilis* (Bhattarai and Shrestha (2009).

The present study also gave similar results with the *Eupatorium odoratum* L. extract giving the maximum inhibition zones with bacterial strains of *Escherichia coli*, *Klebsiellapneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Further the study also analyzed the antibacterial action of the specific active compounds of lignins and proteins by the isolation of compounds from the effective plant extracts. The Protein extracts of *Eupatorium odoratum* L. proved to be the most effective against all the four tested bacteria.

The study carried out by Khare (2008) proves that the medicinal herbs of *Adhatodavastica*, *Zingiberofficinale*, *Piper longum* and *Cinnamomumzeylanicum* proved ideal in ayurvedic formulations and was widely used for curing Urinary Tract Infections. In the present study Ayurvedic formulations as such was not inhibitory in action. It was only bacteriostatic in action. However, plants which are used in ayurvedic formulations showed higher inhibitory action. The current study indicates that Punarnavasavam, ChadrprabhaVatika and Viratradi Kashayam gave a bacteriostatic action when compared to Brihatyadi Kashayam

The use of plant extracts and phytochemicals, both with known antibacterial properties, can be of great significance in therapeutic treatments. Intensive care physicians consider antibiotic resistant bacteria a significant problem in the treatment of diseases. A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds (Doherty, 2010) Medicinal plant extracts, therefore offer considerable potential for the development of new agents effective against infections currently difficult to treat. The finding that the protein extract of *Eupatorium odoratum* L. and *Rotulaaquatica* L. have potential antibacterial activity promises the development of new therapeutic measures to cure urinary infections without much side effects.

The discovery of herbals is further complemented with knowledge on the method of isolation, purification, characterization of active ingredients and type of preparation. The term "herbal drug" determines the part/parts of a plant (leaves, flowers, seeds roots, barks, stems and etc.) used for preparing medicines. Each and every part of the herbs are fully utilized for the different pharmacological action they may produce and made into a range of herbal preparations which serves as a potential future medicine. As per the current study the most effective plant compound was that of protein which could be isolated from plant extracts and used individually or in combination which could be used in ayurvedic formulations, as well as devising of a new medicine which could be more effective in future use better than chemical antibiotics.

## 5. Summary and Conclusion

Natural cure is one of the rising honours of pharmaceuticals wherein Ayurvedic formulations and medicinal plants are identified for their hidden and large extend of unexplored treasure which promises a treatment with lesser side effects and efficacy when compared to chemical antibiotics.

The present study envisages a comparative analysis between the phytochemicals and Ayurvedic formulations to identify the most suitable antibacterial agent to be used against urinary infections. Four strains of bacteria which are leading etiological agents of UTI's identified were *Escherichia coli*, *Klebsiellapneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

The plant extracts of *Eupatorium odoratum* L. and *Rotulaaquatica* L. were effective against bacterial strains of *Escherichia coli*, *Klebsiellapneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The effective plants extracts were further subjected to isolation of lignins and proteins and analyzed for antibacterial action. The protein extract of *Eupatorium odoratum* L. proved ideal as an antibacterial agent.

The ayurvedic formulations of Brihatyadikashayam, Chandraprabhavatika, Punarnavasavam and Viratradi kashayam were tested for its antibacterial activity against the isolates. The Punarnavasavam, Chandraprabhavatika and Viratradi kashayam exhibited bacteriostatic activity against *Klebsiella pneumoniae* and Chandraprabha vatika was effective against *Staphylococcus aureus*.

The plant extracts when used individually proved ideal and served as a better antimicrobial agent against bacteria inhibiting its growth, however Ayurvedic formulations proved to be only bacteriostatic in action. Thus suitable plant extracts could be identified, isolated of its active compounds and used in the treatment of various chronic diseases caused by resistant microbes.

Man's quest for better medicines, search for biomolecules of therapeutic value will continue since today's medicines finance tomorrow's miracles.

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