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An *In-Vitro* Study to Evaluate the Antimicrobial Activity of Polyherbal Combination Against Escherichia Coli in The Management of Mutrakrichra (Urinary Tract Infection)

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ABSTRACT
Millions of people worldwide experience urinary tract infections (UTIs), which are among the most prevalent bacterial infections. UTIs are a common health issue, particularly among women and certain populations. Escherichia coli being the most frequent pathogen, causing 75-90% of UTIs. Timely diagnosis, appropriate treatment, and preventive measures can help reduce the burden of UTIs and minimize complications associated with these infections. In order to address this issue, classical formulations are mentioned in ayurvedic texts. In the current study, Hydroalcholic extracts of Emblica officinalis (Amalaki) and Berberis aristata (Daruharidra) were prepared. The assessment of Antibacterial activity is carried out to determine Minimum inhibitory concentration (MIC) of the extracts and prepared polyherbal combination and further confirmed by determining zone of inhibition. The combination of Amalaki, Daruharidra with honey shows significant inhibition of E.coli which is comparable with Tab. Norfloxacin. This study suggests that Emblica officinalis (Amalaki) and Berberis aristata (Daruharidra) can be used in Mutrakrichra associated with burning micturition (mutradaha) w.s.r. to Urinary tract Infection.
Keywords- *Urinary tract infection, Mutrakrichra, Anti-bacterial activity, Emblica officinalis, Berberis aristata, Honey, E. coli*

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INTRODUCTION

Urinary tract infection is characterized by the invasion, persistence, and proliferation of bacteria within the urinary tract. It is more common in females than males due to shorter urethra which allows the bacteria quick access to the bladder. The most frequent pathogen in uncomlicated UTIs is Escherichia coli, followed by Klebsiella pneumonia. [1] Urinary tract infections (UTIs) can be classified into two types: infection of the lower urinary tract (cystitis) and that of the upper urinary tract (pyelonephritis). Cystitis, the most common type of UTI, involves an infection of the urinary bladder. It manifests with bothersome symptoms such as frequent and urgent urination, a burning sensation during urination, cloudy or bloody urine, and discomfort in the lower abdomen. Pyelonephritis is a more severe form of UTI that affects the kidneys. It often originates as a lower UTI but can ascend and involve the kidneys. Pyelonephritis presents with more systemic symptoms, such as fever, pain in the back or flank area, as well as nausea and vomiting. Prompt medical attention is crucial for pyelonephritis to prevent complications. Acharya Charak[3,5]& Sushruta[4] have explained Mutrakrichra under Mutravahasroto Dusthi Vikara. Ayurveda views Mutrakrichra as a result of the imbalance of doshas (biological humors) in the body, particularly the Vata and Pitta doshas. Dietary and lifestyle factors, improper hygiene practices, and emotional stress can contribute to the development of Mutrakrichra. The contemporary medical approach for managing UTIs primarily involves the use of antibiotics. In routine management, Tab. Norfloxacin is commonly used which may not be a suitable antibiotic in all the cases. While Ayurvedic texts offer numerous classical references to drugs for Mutrakrichhra (urinary disorders), there is a scarcity of clinical studies conducted in this area. It is essential to scientifically validate the antimicrobial activity of the studied drug using scientific parameters. *Emblica*

officinalis (Amalaki) and *Berberis aristata* (Daruharidra)along with honey is mentioned in Charak Samhita under Mutrakrichra chikitsa.[5] *Berberis aristata* (Daruharidra), contains alkaloids, flavonoids, tannins, and other phytochemicals that contribute to its medicinal properties.[6] Berberine, an alkaloid found among its various constituents, exhibits potent antimicrobial activities. Out of its various constituents, the alkaloid berberine exhibits the highest level of antimicrobial activity. [7] *Emblica officinalis* (Amalaki) comprises a range of chemical compounds including tannins, alkaloids, polyphenols, gallic acid, ellagic acid, emblicanin A and B, phyllembein, quercetin, ascorbic acids, as well as various vitamins and minerals.[8] *Emblica officinalis* (Amalaki) exhibits strong antimicrobial properties that effectively combat various bacterial pathogens.[9] The single and combined drugs were tested for their activity against E.coli in comparison with Tab. Norfloxacin in invitro study. The Minimum inhibitory concentration (MIC) was obtained using micro-broth dilution method and the Antibacterial activity of the test extracts was assessed by the agar well diffusion technique. Results were compared with Tab. Norfloxacin. The study was aimed to ascertain the efficacy of polyherbal combination in Mutrakrichra w.s.r. to Urinary tract Infection. Objectives of the study were to determine the anti-bacterial activity of *Emblica officinalis* (Amalaki) and *Berberis aristata* (Daruharidra) and their combination with honey through Minimum inhibitory concentration and Zone of Inhibition and to compare the results with marketed product (Tab. Norfloxacin).

MATERIAL AND METHODS

Drug Collection: The raw drug *Emblica officinalis* (Amalaki) and *Berberis aristata* (Daruharidra) were collected from authenticated supplier and got authentified. The fruit of *Emblica officinalis* (Amalaki) and root of *Berberis aristata* (Daruharidra) were taken.

Extraction: The Soxhlet extraction method was adopted for this study. 50 gms of dried Amalaki fruit powder was taken and packed in filter paper and then soaked in 500 ml solution of ethanol and water (1:1) for a while and placed in Soxhlet thimble. 5 cycles were run till the solvent became transparent and then the system was allowed to self-cool. After that, the solution was filtered using Whatman filter paper No.1 and the extract was collected and reduced in a hot water bath for 6 hrs. The semisolid extract was collected and weighed. Similarly, the extract of Daruharidra root was obtained. Each extract was preserved in an air-tight glass container.

Microorganism used for Study: The species of **Escherichia coli (E. coli)** have been used for the study.

Preparation of culture medium and inoculation: The bacteriological media consisted of nutrient broth and nutrient agar. The media were autoclaved at 120 °C for 15 minutes to sterilize them. 15 ml of culture medium were dispensed into pre-sterilized petridishes in the laminar air flow under aseptic conditions to produce a uniform depth of 4 mm. The microbial cultures were injected using a spread plating technique after the medium had solidified.

Minimum Inhibitory Concentration (MIC) Determination: The minimum inhibitory concentration (MIC) was determined using micro-broth dilution method using Nutrient broth media.[10] Ten test extracts of varied concentrations were created using double-strength broth as the negative and positive controls, respectively, in pre-sterilized tubes. One uninoculated and one inoculated control were kept. The tubes were inoculated with standardized inoculumn suspension (0.5 McFarland standard) and incubated at 370C for 24 hr. Minimum inhibitory concentration were noted as the lowest concentration of extract showing no discernible microbial growth in the broth media.

An Antibacterial assay using agar well diffusion method: The antibacterial activity of the test extracts was assessed by the agar well diffusion method. [11,12] First, the stock cultures of bacteria were revived by inoculating in broth media and incubated at 37°C for 24 hours in order to perform an antibacterial study. Under aseptic circumstances, an aliquot (0.1 ml) of the bacterial suspension (0.5 McFarland standard) was dispersed uniformly in each plate. The suspension in each plate was given 20–25 minutes to dry. The test samples were then placed in wells with a 6 mm diameter that had been created in the solid medium using a sterile cork borer. For 24 hours, all of the plates were incubated at 37°C. The antibacterial activity of each extract was determined by measuring the diameter of the zone of inhibition around each well. Each extract was tested against Escherichia coli in three replications. The results were compared with the marketed formulation.

RESULTS

The minimum inhibitory concentration (MIC) was determined using micro-broth dilution methods by 0.5 McFarland standard shown in Table 1.The Antibacterial activity of samples were evaluated by Agar well diffusion method. The zone of inhibition is measured as shown in Table 2.

Table 1. Minimum Inhibitory Concentration value of Samples

Sample	Concentration[mg/ml]
Daruahidra	120 ± 10.75
Honey	110± 9.15
Amalaki	100± 9.05
Marketed formulation (Tab. Norfloxacin 400)	53.33± 5.01
Daruahidra + Amalaki+ Honey	24+ 22+ 20

Table 2. Antibacterial activity (Zone of Inhibition - ZOI) of different extracts

Zone of Inhibition

Sample	Concentration[mg/ml]	Diameter [cm]
Daruahidra	120	1.8±0.5
Honey	110	1.5±1.5
Amalaki	100	2.5±0.58
Norfloxacin	120	5.5±2.1
Combination of Herbal Extract (Daruahidra + Amalaki+ Honey)	24+22+20	2.4±0.5

DISCUSSION

Nature is a generous source of compounds that hold potential for preventing infections. Herbal medications have experienced a renewed interest in recent years, largely due to their perceived advantages over synthetic pharmaceuticals. These advantages include a lower incidence of adverse reactions and the relatively reduced cost associated with plant preparations which makes the search for natural therapeutics an appealing option. The polyherbal combination of *Emblica officinalis* (Amalaki) and *Berberis aristata* (Daruharidra) with honey is mentioned as a remedy for Pittaja Mutrakrichra in Ayurvedic classics. In this study, minimum inhibitory concentration and Zone of inhibition of the polyherbal combination were assessed. Hydroalcoholic extract of drugs was prepared by Soxhlet method. The minimum inhibitory concentration (MIC) expressed in mg/ml was determined using micro-broth dilution methods. The Minimum inhibitory concentration (MIC) of Daruharidra is 120 ± 10.75, Aamlaki is 100± 9.05, Honey is 110± 9.15 and their combination is 24+ 22+ 20. The Minimum inhibitory concentration (MIC) of Tab. Norfloxacin is 53.33± 5.01. The anti-bacterial activity was evaluated by agar well diffusion method. The zone of inhibition of Amalaki is 2.5±0.58 cm, Daruharidra is 1.8±0.5 cm and the combination of herbal extracts with honey is 2.4±0.5 cm. The Zone of inhibition of Tab. Norfloxacin is 5.5±2.1 cm. The combination of Amalaki, Daruharidra with honey shows the maximum inhibition and antimicrobial activity is well established in comparison with Tab. Norfloxacin. *Berberis aristata* (Daruharidra) and *Emblica officinalis* (Amalaki) are two herbal ingredients known for their medicinal properties. While these herbs have been traditionally used for various purposes, including antimicrobial activity.[13]The antimicrobial activity of *Berberis aristata* (Daruharidra or Indian Barberry) extract may be attributable to the presence of secondary metabolites, particularly Berberine an isoquinolone alkaloid, with a bright yellow hue.[14] *Emblica officinalis* (Amalaki or Indian Gooseberry) has been widely studied for its antimicrobial properties. It contains various constituents, including flavonoids (such as quercetin), ascorbic acid (vitamin C), gallic acid, alkaloids (phyllantine, phyllantidine), and hydrolysable tannins (emblicanin A and B). Fruits of *Emblica officinalis* are the richest source of Vitamin C, tannin and flavonoids, etc.[15] The constituents present in *Emblica officinalis* have been linked to antimicrobial activity against a broad spectrum of bacteria.[16] The combination of Amalaki, Daruharidra with honey shows nearer efficacy to marketed product (Tab. Norfloxacin). *Berberis aristata* (Daruharidra) has rukshaguna, tikta rasa and ushna virya. It is also useful in Prameha and pacifies Ruja and kandu.[17] "Mutrasaya kledavahanam"Mutra is the sthana for kleda.[18]Urinary stasis leads to accumulation of kleda in body. Kleda known to promote growth of krimi. So the tikta rasa, ruksha guna and ushna virya of Daruharidra helps to reduce kleda thereby rendering the environment unsuitable for the growth of pathogens that thrive on kleda. *Emblica officinalis* (Amalaki) has Amla pradhan Panch rasa, Madhura vipak and sheeta virya. It has laghu, ruksha and sara guna. It is tridhoshhara, due to Amla rasa it is Vatashamak, Pittashamak due to Madhura and Sheeta guna

and Kapha shamak due to Ruksha and kashaya rasa.[19]Amalaki is diuretic (mutrala) and anulomana due to its sara guna and relieves burning micturition (mutradaha). In the previous studies, it was observed that the methanolic stem extracts of B. aristata (Daruharidra) demonstrated antibacterial efficacy against S. pneumonia, Nocardia sp., and E. coli.[20] The anti-microbial activity of hydroalcoholic extract of Daruharidra against E.coli and other pathogens was also reported.[21]Amalaki has been studied for its antibacterial activity against UTI-causing bacteria.[22,23] Honey (Madhu) exhibits anti-microbial activity against many organisms including Salmonella, Shigella, E.coli, H.pylori.[24] Parul Institute of Ayurved had successfully conducted research on antibacterial effect of herbal fumigation kwath on Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa [25] The therapeutic potential of *Emblica officinalis* (Aamlaki) and *Berberis aristata* (Daruharidra) in Mutrakrichra as mentioned in ayurvedic texts is strongly supported by this groundbreaking study, which builds upon a robust body of previous research. This study further substantiates their efficacy and highlights their tremendous promise for various therapeutic applications.

CONCLUSION

This groundbreaking study sheds light on the extraordinary antimicrobial properties of *Emblica officinalis* (Aamlaki) and *Berberis aristata* (Daruharidra), unveiling their potential as natural remedies for Mutrakrichra associated with mutradaha, particularly in the context of urinary tract infections. These findings pave the path for the integration of these herbal extracts into targeted treatment strategies, offering a promising solution to combat this common and concerning condition.

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