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SHORT COMMUNICATION

An *In Vitro* Study for Evaluating the Antimicrobial Efficacy 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 rank among the most widespread bacterial infections. UTIs are a common health issue, particularly among women and certain populations. *Escherichia coli* is 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, hydroalcoholic extracts of *Emblica officinalis* (*Amalaki*) and *Berberis aristata (Daruharidra)* were prepared. The antimicrobial activity of the extracts and their combination was evaluated by establishing their Minimum Inhibitory Concentration (MIC) and measuring the zone of inhibition. The combination of *Amalaki* and *Daruharidra* with honey shows significant inhibition of *E. coli* which is comparable with tablet Norfloxacin. This study suggests that *Emblica officinalis* (*Amalaki)* and *Berberis aristata* (*Daruharidra)* can be used in *Mutrakrichra* associated with *Mutradaha* in the context of urinary tract infections.

Keywords: Anti-bacterial Activity, *Berberis aristata*, *Emblica officinalis*, *Escherichia coli*, Honey, *Mutrakrichra*, Urinary Tract Infection

1. 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 uncomplicated UTIs is *Escherichia coli*, followed by *Klebsiella pneumonia*[1]. Urinary tract infections (UTIs) can be categorized as either lower urinary tract infections, also known as cystitis, or upper urinary tract infections, which are referred to as 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[2]. Prompt medical attention is crucial for pyelonephritis to prevent complications.

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Acharya Charak[3] and *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 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 are 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]. 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 Ellagic acid, Amlaic acid, Phyllantine, Phyllantidine, Zeatin, gallic 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 *in vitro* study. The micro-broth dilution method was used to determine the Minimum Inhibitory Concentration (MIC) and the Agar well diffusion technique was used to evaluate the test extracts' antibacterial activity. Results were compared with Tab. Norfloxacin.

The primary aim of this research was to establish the effectiveness of a polyherbal combination in managing *Mutrakrichra* with respect to urinary tract infections.

The purpose of this research was to assess the antibacterial effects of *Emblica officinalis* (*Amalaki*) and *Berberis aristata* (*Daruharidra*), both individually and in combination with honey. This evaluation was carried out using methods such as Minimum Inhibitory Concentration and Zone of Inhibition. The outcomes were then compared with those of a commercially available product (Tablet Norfloxacin) for reference.

2. Materials and Methods

2.1 Drug Collection

The raw drugs *Emblica officinalis* (*Amalaki*) and *Berberis aristata (Daruharidra)* were collected from an authenticated supplier and were authentified. The fruit of *Emblica officinalis (Amalaki)* and the stem of *Berberis aristata* (*Daruharidra)* were taken.

2.2 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 a 500 ml solution of ethanol and water (1:1) for a while and placed in a Soxhlet thimble. 5 cycles were run till the solvent became transparent and then the system was allowed to self-cool. The solution was then filtered through 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* stem was obtained. Each extract was preserved in an air-tight glass container.

2.3 Microorganism used for Study

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

2.4 Preparation of Culture Medium and Inoculation

The bacteriological media consisted of nutrient broth and nutrient agar. To sterilize the media, they were autoclaved at 120°C for 15 minutes. 15 ml of culture medium was administered into pre-sterilized Petri dishes in the laminar airflow under aseptic conditions to produce a uniform depth of 4 mm. After the medium had solidified, the microbial cultures were injected using a spread plating procedure.

2.5 Minimum Inhibitory Concentration (MIC) Determination

The Minimal Inhibitory Concentration (MIC) was determined using the 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 infused with a consistent suspension of inoculum (at a 0.5 McFarland standard) and then kept in an incubator at 37°C for 24 hours. The Minimum inhibitory concentration was determined as the lowest extract concentration at which no observable microbial growth was evident in the liquid culture medium.

2.6 An Antibacterial Assay using Agar Well Diffusion Method

The agar well diffusion method was used to assess the antibacterial activity of the test extracts[11-12]. Initially, the bacterial stock cultures were revitalized by introducing them into liquid broth media and subsequently incubated at 37°C for a duration of 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. The plates were set aside in an incubator at 37°C for a duration of 24 hours. The antibacterial efficacy of each extract was assessed by measuring the diameter of the inhibition zone surrounding each well. Each extract was tested against *Escherichia coli* in three replications. The results were compared with the marketed formulation.

3. Results

The Minimum Inhibitory Concentration (MIC) was determined using micro-broth dilution assay by 0.5 McFarland standard shown in Table 1. The Antibacterial activity of samples was evaluated by the Agar well diffusion method. The measurement of the inhibition zone is illustrated 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

4. 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 *Berbers 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 of *Daruharidra* is 120 ± 10.75, *Amalaki* 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 Inhibition zone 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. 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].

Table 2. Antibacterial activity of various 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

Emblica officinalis (*Amalaki* or Indian Gooseberry) has been widely studied for its antimicrobial properties. It contains various constituents including tannins, flavonoids such as quercetin, polyphenols like ellagic acid, gallic acid, alkaloids, and essential fatty acids. Among these, the phenols are aromatic compounds known for their remarkable antioxidant and antimicrobial properties. Notably, ellagic acid demonstrates anti-inflammatory properties, while gallic acid possesses potent antioxidant and antimicrobial attributes. Fruits of *Emblica officinalis* are the richest source of vitamin C, tannins flavonoids, etc[15]. Collectively, all the phytoconstituents present in *Emblica officinalis* have been linked to antimicrobial activity against a broad spectrum of bacteria[16].

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 action of hydroalcoholic extract of *Daruharidra* counter to *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 *Shigella, E. coli, Salmonella,* and *H. pylori*[24].

Parul Institute of Ayurved has successfully conducted research on the antibacterial effect of herbal fumigation *Kwath* on *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*[25].

The therapeutic potential of *Emblica officinalis* (*Amalaki*) and *Berberis aristata* (*Daruharidra*) in *Mutrakrichra* as mentioned in *Ayurvedic* texts is strongly supported by this 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.

5. Conclusion

This ground breaking 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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