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A STUDY ON THE EFFECT OF METHANOLIC EXTRACTS OF *Maranta arundinacea* LEAVES AGAINST URINARY TRACT INFECTION CAUSING PATHOGENS

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Urinary Tract Infection (UTI) has become a major socio-economic burden because of the multi-drug resistance and the high frequency of repeated use. The goal of the research paper is to describe the use of natural therapies in the treatment and management of UTIs. Even if conventional medicine is less effective than herbal remedies, further research is needed to understand the phytoconstituents and their mechanisms of action that are responsible for the treatment and management of UTI. Many therapeutic benefits, including antibacterial activity, are attributed to *Maranta arundinacea.* Therefore, commonly known UTI pathogens like *Staphylococcus aureus, Bacillus sublitis, Enterococcus sp., Salmonella paratyphi, Pseudomonas aeroginosa, Proteus mirabilis, Escherichia coli, Klebsiella pneumonia, Salmonella typhi,* and *Enterobacter sps*. were evaluated by phytochemical analysis and *in vitro* antimicrobial activity of this plant The antibacterial activity was studied by dilution and diffusion assays. The results of this study conclude that arrowroot methanolic extract can inhibit the growth of pathogens causing UTI with the most effective concentration is 80 µg/ml.

Keywords: Urinary tract infection; phytochemical analysis; antibacterial activity; *Maranta arundinacea L.*; Arrowroot.

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1. INTRODUCTION

A urinary tract infection (UTI) can affect the kidneys, urinary bladder, and, most commonly, the lower urinary tract system, which includes the bladder and urethra. UTIs can affect both men and women. The chances of UTI are very high when the number of causative agent increases above 10,000 CFU/mL in a urine culture or urine analysis [1]. According to research, the most common causes of UTIs are microbial infections by *Escherichia coli*, *Enterococcus faecalis*, and *Klebsiella sp*. Signs and symptoms of UTIs include painful urination, also known as dysuria, hematuria, urinary urogency, burning micturition, frequent urination, nausea, and vomiting. Antibiotics like trimethoprim, sulfamethoxazole, quinolone etc act as the first choice of the drug are used worldwide. Uropathogens are pathogens which are present in the gut and contaminate the peri-urethral area during uncomplicated UTIs. Such pathogens present in urethra move to the bladder where adhesion molecules and pili help them to invade and colonize the superficial umbrella cells. Host immune response in the form of inflammation and neutrophilic infiltration in the infected area helps to phagocytose the microbes [2]. However, some microbes multiply and make biofilms in the urinary bladder upon the evasion of the immune system. Different types of toxins and proteases from bacteria stimulate the host cell damage, and survival of bacteria during the release of vital nutrients which promote the movement of microbes to the kidneys. Colonized bacteria in the kidneys cause host tissue destruction due to the release of toxins from bacteria and bacteremia might occur if the pathogen crosses the tubular epithelial barrier in the kidneys. On the other hand, in complicated UTIs, uropathogens reach the bladder as in uncomplicated UTI. But during complicated UTI, the bladder defensive environment must be compromised to cause the infection [3]. Medicinal plants have been used since ancient times, because they have beneficial effects, to treat and control various disorders. Due to fewer reported side effects, cost effectiveness, easy availability, lack of bacterial resistance and tolerance towards the patients with UTI even at the start of the 21st century, medicinal plants have gained more and more popularity as well as reliability worldwide [4].

Herbal formulation involves use of fresh or dried plant parts. Therefore, proper and correct identification of the plant material is very much essential for their evaluation and standardisation, correct identification of the material is an essential prerequisite to ensure reproducible quality and will contribute immensely to its safety and efficacy. Herbal drugs are safe, economical, and easy to use. The major advantage of these herbal drugs is that bacteria have not developed resistance against them [5-10]. The exact mechanisms of medicinal herbs and their phytochemical constituents that are responsible for the effect on UTI are still to be investigated. Further research is needed to elucidate clearly the mode of action of these phytochemicals. Additional studies are needed to confirm the phytoconstituents that are responsible for the treatment of UTI.

The arrowroot plant *M. arundinacea* L. is identified to possesses phytochemicals which make them medically important in exhibiting antidiarrheal, probiotic, antiulcer, antioxidant, antimicrobial, vibriocidal and immunostimulatory effects [11]. But not many research regarding antimicrobial effect of *M. arundinacea* against common UTI are not recorded. Based on the reasons, the researcher is interested to investigate the antimicrobial effect of arrowroot methanolic extract against *Staphylococcus aureus, Bacillus sublitis, Enterococcus sp., Salmonella paratyphi, Pseudomonas aeroginosa, Proteus mirabilis, Escherichia coli, Klebsiella pneumonia, Salmonella typhi* and *Enterobacter* sps.

Fig. 1. *Maranta arundinacea L.* Leaves

2. MATERIALS AND METHODS

2.1 Plant Sample Collection

Leaves from *Maranta arundinacea* were the plant material used in this investigation. These plants came from a variety of locations in Kerala's Palakkad. The leaves are the area that is used to examine antibacterial activity. Primary attention is placed on leaf authentication. According to environmental responses, the arrowroot stem is upright and has various-sized leaves at its top. Typically, they can be up to 12 cm across and have a pretty substantial size. Leaf blade, pulvinus, petiole, and sheath are the parts of the leaves [12].

2.2 Macroscopic and Powder Analysis

Fresh plant materials were employed for macroscopic and organoleptic analysis in order to authenticate and standardise the products. Macroscopic evaluation of *M. arundinacea* was done, and certain macroscopic characteristics were noticed, including surface, form, size, colour, venation, phyllotaxy, and length of the petiole and leaf. A physical characterisation of dried whole plant powder was also conducted.

2.3 Plant Extraction

Using a soxhlet apparatus, methanol was employed as the extraction solvent for 24 hours at a temperature of 56°C on powdered *Maranta arundinacea* leaf. The extracts were concentrated by running them through a rota evaporator, and the solvent was collected. This is the stock that is kept in airtight containers at 4°C.

2.4 Screening of Phytochemicals

The methanolic extract's qualitative phytochemical analysis was conducted using the same procedures employed by Jayakumar and Suganthi [13]. Tests were run for steroids, terpenoids, flavonoids, coumarins, emodin, alkaloids, proteins, phlobatannin, leucoanthocyanin, cardiac glycosides, anthraquinones, phenol, and xanthoprotein. The existence of phytochemical components that might be the cause of the antibacterial activity was checked in the leaf and rhizome extracts of *Maranta arundinacea* L. There have been few investigations done on *M. arundinacea* L. methanolic extract. An initial test for identifying the phytoconstituents is the screening of phytochemicals or the qualitative analysis. It helps explain how the plant acquired its antimicrobial qualities.

2.5 Bacterial Inoculation Preparation

The microbiology department at the RMMCH, (Raja Muthiah Medical College and Hospital) in Chidambaram, Tamil Nadu, provided pathogenic strains of *Staphylococcus aureus, Bacillus sublitis, Enterococcus* sp., *Salmonella paratyphi, Pseudomonas aeroginosa, Proteus mirabilis, Escherichia coli, K pneumonia, S typhi*, and *Enterobacter* sp. The bacterial strains are cultivated in MHA plates and kept at 37°C for 24 hours as part of the experiment.

2.6 Dilution Methods

The dilution techniques consist of MBC and MIC (Minimum Inhibitory Concentration) (Minimum Bactericidal Concentration). The arrowroot extract concentrations of 20µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, and 100 µg/ml were the independent variables in this study. The proliferation of pathogens is the dependent variable in this study.

For the dilution method, the experiment is carried out by preparing seven tubes of bacterial suspensions, which contain *Staphylococcus aureus, Bacillus sublitis, Entertutuococcus sp., Salmonella paratyphi, Pseudomonas aeroginosa, Proteus mirabilis, Escherichia coli, Klebsiella pneumonia, Salmonella typhi,* and *Enterobacter* sp. in Mueller Hinton Broth (MHB). Ciprofloxacin at a concentration of 10 µg/mL is taken as positive. It is then incubated at 37ºC for 24 hours. The concentration that yields clear tubes are then cultured in Mueller Hinton Agar (MHA) using the streak plate method and then incubated at 37ºC for 24 hours. This is done to determine the sterility of the clear tubes.

2.7 Well Diffusion Method Assay

The experiment is conducted by spreading the bacterial suspension on MHA that has been given 6 wells, then the arrowroot extracts concentrations 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml and positive control are poured into the wells. It is then incubated at 37°C for 24 hours. This is done to determine the inhibitory zone diameter of arrowroot methanolic extracts.

3. RESULTS

The present research work was carried out in the Department of Microbiology, Faculty of Science, Annamalai University. The studies were done for finding the antimicrobial activity of *M. arundinacea L.* on common UTI pathogens. The methanolic leaf extracts of *Maranta arundinacea* L. was investigated for *in vitro* antibacterial activity. The antibacterial activity was assessed by well diffusion and microdilution method. Dry leaves of *Maranta arundinacea* L. were extracted with the methanol as the solvent which is of increasing polarity.

Table 1. Organoleptic properties

Characters	***M. arundinacea* L. leaves**
Fresh plant leaf	
Leaf size	14cm ± 2
Leaf shape	Lanceolate
Leaf margin	Smooth
Petiole	5cm-12cm
Taste	Characteristic
Dried whole plant	
Colour	Dark green
Odour	Characteristic
Taste	Bitter
Texture	Rough, fibrous

Table 2. Qualitative Phytochemical activity test of methanolic extracts of *M. arundinacea* leaves

Phytoconstituents	***M. arundinacea* Leaf extract**
Alkaloids	+
Tannins	+
Saponins	+
Glycosides	+
Steroids	+
Proteins	+
Amino acids	+
Carbohydrates	+
Terpenoids	+

+ present

Table 3. Arrowroot methanolic extract MIC results

Organism			**Concentration µg/ml**				
Gram positive	**Positive**	**negative**	**20**	**40**	**60**	**80**	**100**
Staph aureus	Clear	Turbid	Turbid	Turbid	Slightly Turbid	Clear	Clear
Bacillus sublitis	Clear	Turbid	Turbid	Turbid	Slightly Turbid	Clear	Clear
Enterococcus sp.	Clear	Turbid	Turbid	Turbid	Slightly Turbid	Clear	Clear
Gram negative							
Salmonella paratyphi	Clear	Turbid	Turbid	Turbid	Slightly Turbid	Clear	Clear
Pseudomonas aeroginosa	Clear	Turbid	Turbid	Turbid	Slightly Turbid	Clear	Clear
Proteus mirabilis	Clear	Turbid	Turbid	Turbid	Slightly Turbid	Clear	Clear
Escherichia coli	Clear	Turbid	Turbid	Turbid	Slightly Turbid	Clear	Clear
K pneumonia	Clear	Turbid	Turbid	Turbid	Slightly Turbid	Clear	Clear
S typhi	Clear	Turbid	Turbid	Turbid	Slightly Turbid	Clear	Clear
Enterobacter	Clear	Turbid	Turbid	Turbid	Turbid	Clear	Clear

Staph aureus- Staphylococcus aureus, B. subtilis

Table 4. Arrowroot methanolic extract MBC results

Organism	Groups			
Gram positive	**Positive**	**negative**	**80 µg/ml**	**100 µg/ml**
Staphylococcus aureus	No growth	growth	No growth	No growth
Bacillus sublitis	No growth	growth	No growth	No growth
Enterococcus sp.	No growth	growth	No growth	No growth
Gram negative				
Salmonella paratyphi	No growth	growth	No growth	No growth
Pseudomonas aeroginosa	No growth	growth	No growth	No growth
Proteus mirabilis	No growth	growth	No growth	No growth
Escherichia coli	No growth	growth	No growth	No growth
Klebsiella pneumonia	No growth	growth	No growth	No growth
Salmonella typhi	No growth	growth	No growth	No growth
Enterobacter sp.	No growth	growth	No growth	No growth

3.1 Organoleptic Characteristics

It is a screening test for the standardization and identification of plants. It is easier to verify the validity of studies when plants can be identified. Table 1 displays the characteristics under investigation for the research project and contrasts them with parameters previously established by different researchers. Similar outcomes were obtained.

3.2 Phytochemical Screening

Phytocompounds such as flavonoids, alkaloids, tannins, glycosides, steroids, phenols, cardiac glycosides, and saponins were found during the screening of arrowroot methanolic extract. The plant's antibacterial characteristics were confirmed by a qualitative test that revealed the presence of important phytochemicals.

3.3 Dilution Methods

The concentration of arrowroot methanolic extract started to exhibit clarity at 80 µg/ml, according to the dilution procedure. The bioactive components' MIC was established as the lowest concentration at which pathogenic strain growth in liquid LB medium was inhibited. For 24 hours, the various concentrations were incubated at 37°C. 20 µg/ml, 40 µg/ml, and 60 µg/ml of arrowroot displayed turbidity. Table 2 and Table 3 present a results of the MIC and MBC.

Table 5. Arrowroot methanolic extract inhibitory zone of measurement

Organisms	Zone of inhibition						
Gram positive	**Positive Ciprofloxacin 10 µg/mL**	**Negative**	**20**	**40**	**60**	**80**	**100**
Staphylococcus aureus	20	-	-	10 mm	13mm	14mm	20mm
Bacillus sublitis	18	-	-	10mm	14mm	18mm	19mm
Enterococcus sp.	15	-	-	-	12mm	16mm	18mm
Gram negative							
Salmonella paratyphi	16	-	-	10mm	12mm	15mm	17mm
Pseudomonas aeruginosa	22	-	-	-	12mm	14mm	19mm
Proteus mirabilis	15	-	-	10mm	13mm	17mm	18mm
Escherichia coli	21	-	-	-	13mm	14mm	20mm
Klebsiella pneumonia	14	-	-	-	12mm	14mm	17mm
Salmonella typhi	17	-	-	10mm	14mm	14mm	16mm
Enterobacter sps.	18	-	-	-	13mm	16mm	18mm

3.4 Well Diffusion Method Assay

The diffusion method results showed that the concentration of arrowroot methanolic extracts began to show inhibitory zone in 40 µg/ml with a mean diameter of 10 µg/ml against all the Pathogens except *P. aeruginosa* and *E. coli*. For *P. aeruginosa* and *E. coli* the zone of inhibition starts at 60 µg/ml. As the concentration increases the zone of inhibition also increases. The maximum zone of inhibition is shown against *Bacillus subtilis* with a zone of 18 mm and 19mm against 60 µg/ml and100 µg/ml. All the experiments were done in triplets confirming the antibacterial activity. The results are tabulated in Table 5. The presence of antimicrobial activity is proved because of its phytoconstituents mainly phenols, flavonoids, tannins, alkaloids, steroids, and terpenoids [14].

4. DISCUSSION

Maranta arundinacea L. was used to treat a variety of diarrheal illnesses and was mostly popular as a starchy food. The chemical makeup of elements in methanolic plant extract can be revealed through phytochemical screening, which is also utilised to look for bioactive molecules that could be used to create very beneficial medications.

Secondary plant metabolites like steroids, alkaloids, flavonoids, tannins, phenols, and other aromatic chemicals function as protective macromolecules against microorganisms. Phytochemicals exert antimicrobial activity through different mechanisms. All plant cells often accumulated bioactive substances as secondary metabolites. Different methods are used by secondary metabolites to exert antibacterial action. Total phenolic content and antibacterial activity are correlated favourably [15-17]. The substances having phenolic structures had a significant level of antimicrobial activity. Depending on the concentration utilised, members of this class are known to be either bactericidal or bacteriostatic agents [18]. Tannins have been found to permanently bind to proline-rich proteins, inhibiting the creation of proteins in cells. Herbs that are primarily composed of tannins are astringent in nature and used to treat gastrointestinal illnesses including diarrhoea and dysentery. The phytochemicals prevent the bacteria's ability to synthesise peptidoglycans for their cell walls and prevent the production of proteins. Additionally, they disrupt peptide bonds during the formation of nucleic acids, function as chelating agents, impede metabolic pathways, and stop microorganisms from utilising available nutrients. Some substances lead to the lysis of bacterial cell.

The results showed that the MIC and MBC for arrowroot methanolic extract against MRSA was 100%. This shows that arrowroot methanolic extract affects bacterial growth MRSA. Arrowroot contains a variety of biologically active plant chemicals including flavonoids and terpenoids which in some studies have antibacterial effects [13].

Flavonoids have a number of antibacterial actions, including the suppression of energy metabolism, cytoplasm membrane function, and nucleic acid production. Other strategies include creating covalent bonds and complexes with proteins through non-specific processes like hydrogen bonding and hydrophobic effects [19-21]. Terpenoids can poison a bacterium's structure and membrane functioning. They can also prevent bacterial respiration and ion transfer.

The strength of this research is that this research utilizes two methods in antimicrobial susceptibility testing which are dilution and diffusion tests to determine the MIC, MBC, and inhibitory zone diameter of arrowroot methanolic extract against common UTI pathogens where other researches only measure the inhibitory zone diameter of an extract.

5. CONCLUSION

In conclusion, present study of *M. arundinacea* helps in highlighting the importance of the herb in antimicrobial properties against UTI pathogens. The plant part of the taxa has their own distinct and unique features. The antibacterial activity by various *in vitro* method showed the medical importance of the forgotten plant *M. arundinacea*. The reason for its activity was confirmed by the phytochemical screening which highlights the biocomponents present. Future studies in the research can be by extracting the bioactive molecule, its identification and will help in the future formulation of antibiotics against UTI causing pathogens.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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